

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/01561

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07H21/00 C07B61/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07B C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ✓	WO 95 04160 A (ISIS INNOVATION ; SOUTHERN EDWIN (GB); CUMMINS WILLIAM JONATHAN (GB) 9 February 1995 (1995-02-09) cited in the application page 47; claims 1-15; figure 3	52-54
Y		31-44, 55-58
A		1-30
Y	WO 97 27331 A (DARWIN MOLECULAR CORP ; TABONE JOHN C (US); MULLIGAN JOHN T. (US); H) 31 July 1997 (1997-07-31) page 12 -page 16 page 28 -page 29; figures 9,10; example 16 --- -/--	31-44, 55-58

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&amp;" document member of the same patent family

Date of the actual completion of the international search

15 November 1999

Date of mailing of the international search report

09/12/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Hart-Davis, J

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/01561

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 424 819 A (MILLIPORE CORP) 2 May 1991 (1991-05-02) page 8 page 10 figure 1 & US 5 410 068 A cited in the application ---	31-44, 55-58
X	WO 98 20020 A (KOSTER HUBERT ;LITTLE DANIEL P (US); SEQUENOM INC (US); CANTOR CHA) 14 May 1998 (1998-05-14) claims 1,20-24,83,89,90,100-102 ---	45,49-51
X	WO 98 20019 A (REUTER DIRK ;HIGGINS G SCOTT (DE); LOUGH DAVID M (GB); KOESTER HUB) 14 May 1998 (1998-05-14) page 19, line 5 -page 21, line 23; figures 10-13 ---	45,49-51
A	WO 95 28640 A (UNIV COLUMBIA ;COLD SPRING HARBOR LAB (US); STILL W CLARK (US); WI) 26 October 1995 (1995-10-26) page 5 -page 6 page 31 -page 32 page 51 -page 57 ---	1-30
A	WO 96 30337 A (SMITHKLINE BEECHAM CORP ;GARIGIPATI RAVI SHANKER (US); ADAMS JERRY) 3 October 1996 (1996-10-03) the whole document ---	31-44, 55-58
P,Y	WO 98 31830 A (BRAX GENOMICS LTD ;THOMPSON ANDREW HUGIN (GB); SCHMIDT GUENTER (GB) 23 July 1998 (1998-07-23) claims 1-50; figures 7,8 -----	31-44, 55-58
A		1-30

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/01561

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9504160	A	09-02-1995	AT 159767 T	15-11-1997
			AU 695349 B	13-08-1998
			AU 7269194 A	28-02-1995
			CA 2168010 A	09-02-1995
			CN 1131440 A	18-09-1996
			DE 69406544 D	04-12-1997
			DE 69406544 T	26-02-1998
			DK 711362 T	22-12-1997
			EP 0711362 A	15-05-1996
			EP 0778280 A	11-06-1997
			ES 2108479 T	16-12-1997
			FI 960403 A	29-01-1996
			HU 73802 A	30-09-1996
			JP 9501830 T	25-02-1997
			NO 960370 A	28-03-1996
			US 5770367 A	23-06-1998
WO 9727331	A	31-07-1997	AU 2247397 A	20-08-1997
			CA 2243560 A	31-07-1997
			CN 1212021 A	24-03-1999
			CZ 9802184 A	16-12-1998
			EP 0868535 A	07-10-1998
			HU 9901321 A	28-07-1999
			NZ 331044 A	25-02-1999
			PL 328271 A	18-01-1999
EP 0424819	A	02-05-1991	US 5410068 A	25-04-1995
			DE 69015566 D	09-02-1995
			DE 69015566 T	10-08-1995
			JP 3279371 A	10-12-1991
WO 9820020	A	14-05-1998	AU 5106998 A	29-05-1998
			AU 5198098 A	29-05-1998
			EP 0954612 A	10-11-1999
			EP 0937096 A	25-08-1999
			NO 992168 A	06-07-1999
			NO 992169 A	06-07-1999
			WO 9820166 A	14-05-1998
			AU 5247298 A	29-05-1998
			DE 19782097 T	14-10-1999
			EP 0937097 A	25-08-1999
			NO 992167 A	05-07-1999
			WO 9820019 A	14-05-1998
WO 9820019	A	14-05-1998	US 5900481 A	04-05-1999
			AU 5106998 A	29-05-1998
			AU 5247298 A	29-05-1998
			DE 19782097 T	14-10-1999
			EP 0954612 A	10-11-1999
			EP 0937097 A	25-08-1999
			NO 992167 A	05-07-1999
			NO 992168 A	06-07-1999
			WO 9820166 A	14-05-1998
			AU 5198098 A	29-05-1998
			EP 0937096 A	25-08-1999
			NO 992169 A	06-07-1999
			WO 9820020 A	14-05-1998

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/01561

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9528640	A	26-10-1995	US 5565324 A	15-10-1996
			AU 2292695 A	10-11-1995
			CA 2187792 A	26-10-1995
			CN 1151793 A	11-06-1997
			EP 0755514 A	29-01-1997
			HU 74985 A	28-03-1997
			JP 10502614 T	10-03-1998
			NO 964332 A	03-12-1996
			US 5968736 A	19-10-1999
			US 5789172 A	04-08-1998
WO 9630337	A	03-10-1996	EP 0817771 A	14-01-1998
			JP 11502854 T	09-03-1999
WO 9831830	A	23-07-1998	AU 5570098 A	07-08-1998
			AU 1770499 A	12-07-1999
			WO 9932501 A	01-07-1999



## PATENT COOPERATION TREATY

PCT

NOTIFICATION CONCERNING  
AMENDMENTS OF THE CLAIMS  
  
(PCT Rule 62 and  
Administrative Instructions, Section 417)

From the INTERNATIONAL BUREAU

To:

Mrs I. Diallo  
European Patent Office  
Erhardtstrasse 27  
D-80331 Munich  
ALLEMAGNE

in its capacity as International Preliminary Examining Authority

Date of mailing (day/month/year)

18 January 2000 (18.01.00)

International application No.

PCT/GB99/01561

International filing date (day/month/year)

17 May 1999 (17.05.99)

Applicant

ISIS INNOVATION LIMITED et al

The International Bureau hereby informs the International Preliminary Examining Authority that no amendments under Article 19 have been received by the International Bureau (Administrative Instructions, Section 417).

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Facsimile No. (41-22) 740.14.35

Authorized officer

Olivia RANAIVOJAONA

Telephone No. (41-22) 338.83.38

## PATENT COOPERATION TREATY

PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents  
 United States Patent and Trademark  
 Office  
 Box PCT  
 Washington, D.C. 20231  
 ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

<b>Date of mailing</b> (day/month/year) 18 January 2000 (18.01.00)	
<b>International application No.</b> PCT/GB99/01561	<b>Applicant's or agent's file reference</b> PP/2695 PCT
<b>International filing date</b> (day/month/year) 17 May 1999 (17.05.99)	<b>Priority date</b> (day/month/year) 15 May 1998 (15.05.98)
<b>Applicant</b> SOUTHERN, Edwin, Mellor et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:

08 December 1999 (08.12.99)

☐ in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was

☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland  Facsimile No.: (41-22) 740.14.35	Authorized officer  Olivia RANAIVOJAONA  Telephone No.: (41-22) 338.83.38
---	---

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PP/2695PCT	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 99/ 01561	International filing date (day/month/year) 17/05/1999	(Earliest) Priority Date (day/month/year) 15/05/1998
Applicant  ISIS INNOVATION LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☐ contained in the international application in written form.

☐ filed together with the international application in computer readable form.

☒ furnished subsequently to this Authority in written form.

☒ furnished subsequently to this Authority in computer readable form.

☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

LIBRARIES OF OLIGOMERS LABELLED WITH DIFFERENT TAGS

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☒ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

1  
☐ None of the figures.

# INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/01561

## Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
  
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
  
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

## Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
  
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
  
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
  
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

## FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-30, 42-43 (partially)

A set of labelled compounds (optionally attached to a particulate solid support) wherein different molecules / lots of the set are labelled with different tags.

2. Claims: 31-41, 42-43 (partially), 44, 55-58

Labels, e.g. for oligonucleotide probe analysis, comprising a label attached by a link cleavable to give a charged species for analysis by mass spectrometry, e.g. N-hydroxysuccinimide- (NHS-) or amide-bearing monomethoxytrityl systems.

3. Claims: 45-51

Inserts for use in laser desorption mass spectrometry, the inserts having target surfaces of glass or of an organic polymer.

4. Claims: 52-54

Analysis of nucleic acids comprising oligonucleotide reagents tagged with moieties suitable for analysis by mass spectrometry.

# PATENT COOPERATION TREATY

## PCT

REC'D 08 SEP 2000

WIPO

PCT

### INTERNATIONAL PRELIMINARY EXAMINATION REPORT



(PCT Article 36 and Rule 70)

Applicant's or agent's file reference <b>PP/2695PCT</b>	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. <b>PCT/GB99/01561</b>	International filing date (day/month/year) <b>17/05/1999</b>	Priority date (day/month/year) <b>15/05/1998</b>
International Patent Classification (IPC) or national classification and IPC <b>C07H21/00</b>		
Applicant <b>ISIS INNOVATION LIMITED et al.</b>		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 10 sheets, including this cover sheet.  
  
☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  
  
 These annexes consist of a total of 10 sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☒ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☒ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand  <b>08/12/1999</b>	Date of completion of this report  <b>04.09.2000</b>
Name and mailing address of the international preliminary examining authority:   <b>European Patent Office</b> <b>D-80298 Munich</b> <b>Tel. +49 89 2399 - 0 Tx: 523656 epmu d</b> <b>Fax: +49 89 2399 - 4465</b>	Authorized officer  <b>Döpfer, K-P</b>  Telephone No. +49 89 2399 8547  

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01561

## I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

### Description, pages:

1-46 as originally filed

### Claims, No.:

1-58 as received on 31/07/2000 with letter of 28/07/2000

### Drawings, sheets:

1/8-8/8 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

## II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
- ☐ copy of the earlier application whose priority has been claimed.
  - ☐ translation of the earlier application whose priority has been claimed.
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01561

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

**see separate sheet**

## IV. Lack of unity of invention

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

**see separate sheet**

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☒ all parts.
- ☐ the parts relating to claims Nos. .



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB99/01561

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes:	Claims	11-14,19-21,26-28,35-44,46-48,55-58
	No:	Claims	1-10,15-18,22-25,29,30-34,45,49-54
Inventive step (IS)	Yes:	Claims	26-28,46-48
	No:	Claims	1-25,29-45,49-58
Industrial applicability (IA)	Yes:	Claims	1-58
	No:	Claims	

**2. Citations and explanations**

**see separate sheet**

**VI. Certain documents cited**

**1. Certain published documents (Rule 70.10)**

and / or

**2. Non-written disclosures (Rule 70.9)**

**see separate sheet**

**VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

---

International application No. PCT/GB99/01561

**Re Item I**

**Basis of the report**

**Re Item II**

**Priority**

1. The priority document in respect of the present application is at present not available to the International Preliminary Examination Authority. In consequence the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, the document WO 98 31830 A (BRAX GENOMICS LTD ;THOMPSON ANDREW HUGIN (GB); SCHMIDT GUENTER (GB) 23 July 1998 (1998-07-23) cited in the international search report could become relevant (see also Item VI of this report).

**Re Item III**

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

**Re Item IV**

**Lack of unity of invention**

1. The ISA stated lack of unity of the invention according to Rule 13(1)(2) PCT without inviting the applicant to pay additional fees. The IPEA agrees with the statement of the ISA and considers the following groups of inventions as being present:
  - (i) Method of making a set of labelled compounds and sets of labelled compounds (the labels being cleavable, and the compounds being optionally attached to a particulate solid support) in general (Claims 1-30, 42-43

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

---

International application No. PCT/GB99/01561

partially)

- (ia) further non-unity  
a specific variable mass-tag system based on tritylamides
  - (ii) Labels, e.g. for oligonucleotide probe analysis, comprising a label attached by a link cleavable to give charged species for analysis by mass spectrometry, e.g. N-hydroxysuccinimide- (NHS-) or amide-bearing monomethoxytrityl systems (Claims 31-41, 42-43 (partially), 44, 55-58)
  - (iii) Inserts for use in laser desorption mass spectrometry, the inserts having target surfaces of glass or of an organic polymer.  
(Claims 45-51)
  - (iv) Analysis of nucleic acids comprising oligonucleotide reagents tagged with moieties suitable for analysis by mass spectrometry (Claims 52-54)
2. The groups of inventions are not linked by a common special technical feature contributing to novelty and inventive step so as to form a single inventive concept underlying the present application (see in particular group (iii) as creating the non-unity *a priori* (without taking the prior art into consideration) (Rule 13(1)(2) PCT). Group (ia) is considered originating from the presence of prior art destroying novelty of the general tagging concept (cf Item V of this written opinion), i.e. non-unity *a posteriori* is to be stated.

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Reference is made to the following documents:

D1: WO 95 04160 A (ISIS INNOVATION ;SOUTHERN EDWIN (GB); CUMMINS WILLIAM JONATHAN (GB) 9 February 1995 (1995-02-09) cited in the application

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/01561

- D2: WO 97 27331 A (DARWIN MOLECULAR CORP ;TABONE JOHN C (US); MULLIGAN JOHN T (US); H) 31 July 1997 (1997-07-31)
- D3: EP-A-0 424 819 (MILLIPORE CORP) 2 May 1991 (1991-05-02) & US 5 410 068 A cited in the application
- D4: WO 98 20020 A (KOSTER HUBERT ;LITTLE DANIEL P (US); SEQUENOM INC (US); CANTOR CHA) 14 May 1998 (1998-05-14)
- D5: WO 98 20019 A (REUTER DIRK ;HIGGINS G SCOTT (DE); LOUGH DAVID M (GB); KOESTER HUB) 14 May 1998 (1998-05-14)
- D6: WO 95 28640 A (UNIV COLUMBIA ;COLD SPRING HARBOR LAB (US); STILL W CLARK (US); WI) 26 October 1995 (1995-10-26)
- D7: WO 96 30337 A (SMITHKLINE BEECHAM CORP ;GARIGIPATI RAVI SHANKER (US); ADAMS JERRY) 3 October 1996 (1996-10-03)
- D8: WO 98 31830 A (BRAX GENOMICS LTD ;THOMPSON ANDREW HUGIN (GB); SCHMIDT GUENTER (GB) 23 July 1998 (1998-07-23)
- D9: WO-A-93/06121 (The document was not cited in the international search report. A copy of the document is appended hereto.)

2. The present application relates to the different subject-matters as mentioned under Item IV of this written opinion

2.1 Novelty (Article 33(2) PCT)

Subject-matter (i) and (ia): D9 discloses tagging methods for oligomers including the use of cleavable tags (in particular peptides and oligonucleotides). This is considered novelty destroying for the subject-matter of present claims 1-10, 15-18, 22-25, 29 and 30. D9 does not mention explicitly the use of tags which are useful as mass tags. Present claims 11-14, 26-28 can be considered novel in the view of the prior art cited.

Subject-matter (ii): Independent claim 31 pertains to a method of providing a labelled (or tagged ) oligonucleotide or nucleic acid, and removing the label by cleavage which is subjected to MS. D2 (cf e.g. example 15) discloses such a method comprising the steps as claimed. The use of labelled primer molecules is also mentioned. Thus , present claims 31-34 are considered not novel.

None of the prior art documents cited in the ISR discloses subject-matter which

can be regarded as identical with that of present claims 35-41, 42-43 (partially), 44, 55-58.

Subject-matter (iii): D4 and D5 disclose immobilised nucleic acids on solid supports and methods. Present claims 45, 49-51 relate to a product *per se*, i.e. an immobilised compound and its support (designated as insert for MS). The subject-matter of the above mentioned present claims is directed to planar inserts and direct attachment of the immobilised compounds to the support. Direct immobilisation is understood in that the item to be immobilised is attached to the solid support without using linker or spacer molecules which are separately bound to the support prior to the immobilisation of the desired molecule. The disclosure of documents D4 and D5 includes such items. Therefore, novelty cannot be acknowledged for these claims.

The particular embodiments of present claims 46-48 are considered novel in view of the available prior art.

Subject-matter (iv): D1 discloses methods of tagging oligonucleotide molecules with labels and subsequent decoding via MS analysis. Accordingly, the subject-matter of present claims 52-54 is not novel because the system as claimed in said claims must have been used.

## **2.2 Inventive step (Article 33(3) PCT)**

The novel subject-matter of present claims 11-14 appears not to involve an inventive step because the contribution of the subject-matter of these claims with respect to the technical teachings of the prior art (e.g. D3-D5) does not lead the skilled person to the conclusion that either a particularly advantageous or surprising solution to a technical problem (i.e. to provide an alternative method for making a set of labelled compounds which can be deciphered, in the present case to introduce mass tags which allow easy deciphering of members of a combinatorial library) has been found.

The novel subject-matter relating to the mass-tags (35-41, 42-43 (partially), 44, 55-58) is considered lacking an inventive step for the following reasons:

Mass-tagging for deconvoluting and deciphering sequences is known from the prior art (see documents cited *supra*). Taking D2 as representing the closest prior art, the problem underlying the present application is to be regarded as to provide further labels which are suitable as mass tags. The solution are the claimed substituted monomethoxytrityl compounds. D3 discloses labels with dimethoxytrityl groups which are obviously suitable as mass tags. The skilled person dealing with the problem posed would have come to the claimed solution by combining the teachings of D2 and D3 (combination with D1 would have lead to the same result). The present application discloses the use monomethoxytrityl tags. It is a matter of normal experimentation to modify the degree of methoxy substitution. The presently claimed monomethoxy moieties appear neither to have any particular advantages over the dimethoxy compounds not seem they to exhibit surprising effects which could create presence of inventive step. The particularly designed inserts of claims 46-48 appear to be inventive over the prior art as cited.

Any of the claimed libraries of the application do not involve an inventive step either, because the purpose of a library is in principle to screen for substances possessing a desired property. Since none of the libraries contains structural elements which are considered novel and inventive, it cannot regarded to require inventive activity to create a collection of compounds forming said libraries.

### 2.3 Industrial applicability (Article 33(4) PCT)

The subject-matter of present claims 1-58 appear to comply with the requirements of industrial applicability as stipulated in Article 33(4) PCT.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

---

International application No. PCT/GB99/01561

**Re Item VI**

**Certain documents cited**

Certain published documents (Rule 70.10)

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO-A-98/31830	23.07.1998	15.01.1998	15.01.1997
			28.08.1997
			19.12.1997

When entering the European phase the disclosure of the above mentioned document is to be taken into consideration for the assessment of novelty under Article 54(3) EPC.

**Re Item VII**

**Certain defects in the international application**

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2, D4-D9 are not mentioned in the description, nor are these documents identified therein.

STEVENS HEWLETT  
& PERKINS  
RECEIVED

- 6 DEC 1999

## PATENT COOPERATION TREATY

WO 99/60007  
PCT/GB99/01561

From the INTERNATIONAL BUREAU

PCT

DIA

FILE  
No.

NOTICE INFORMING THE APPLICANT OF THE  
COMMUNICATION OF THE INTERNATIONAL  
APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:

PENNANT, Pyers  
Stevens Hewlett & Perkins  
1 Serjeants' Inn  
Fleet Street  
London, Greater London EC4Y 1NT  
ROYAUME-UNI

Date of mailing (day/month/year)

25 November 1999 (25.11.99)

Applicant's or agent's file reference

PP/2695 PCT

OF

## IMPORTANT NOTICE

International application No.

PCT/GB99/01561

International filing date (day/month/year)

17 May 1999 (17.05.99)

Priority date (day/month/year)

15 May 1998 (15.05.98)

Applicant

ISIS INNOVATION LIMITED et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
- AU,CN,EP,IL,JP,KP,KR,US

In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:

AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CU,CZ,DE,DK,EA,EE,ES,FI,GB,GD,GE,GH,GM,HR,  
HU,ID,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MD,MG,MK,MN,MW,MX,NO,NZ,OA,PL,PT,RO,RU,  
SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,UA,UG,UZ,VN,YU,ZA,ZW

The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on 25 November 1999 (25.11.99) under No. WO 99/60007.

## REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, a demand for international preliminary examination must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

## REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the national phase, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO  
34, chemin des Colombettes  
1211 Geneva 20, Switzerland

Authorized officer

J. Zahra

Facsimile No. (41-22) 740.14.35

Telephone No. (41-22) 338.83.38



## PATENT COOPERATION TREATY

50

From the  
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

PCT

To:

PENNANT, Pyers  
Stevens Hewlett & Perkins  
1 Serjeants' Inn  
Fleet Street  
London EC4Y 1NT  
GRANDE BRETAGNE

RECEIVED

-5 SEP 2000

DIA

FILE  
No.

NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT  
(PCT Rule 71.1)

Date of mailing  
(day/month/year)

04.09.2000

Applicant's or agent's file reference  
PP/2695PCT

## IMPORTANT NOTIFICATION

International application No.  
PCT/GB99/01561

International filing date (day/month/year)  
17/05/1999

Priority date (day/month/year)  
15/05/1998

Applicant

ISIS INNOVATION LIMITED et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

## 4. REMINDER

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA



European Patent Office  
D-80298 Munich  
Tel. +49 89 23399 - 0 Tx: 523656 epmu d  
Fax: +49 89 23399 - 4465

Authorized officer

Gallego, A

Tel. +49 89 23399-8102




## PATENT COOPERATION TREATY

## PCT

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PP/2695PCT		<b>FOR FURTHER ACTION</b>		See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)
International application No. PCT/GB99/01561		International filing date (day/month/year) 17/05/1999		Priority date (day/month/year) 15/05/1998
International Patent Classification (IPC) or national classification and IPC C07H21/00				
Applicant ISIS INNOVATION LIMITED et al.				
<p>1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.</p> <p>2. This REPORT consists of a total of 10 sheets, including this cover sheet.</p> <p><input checked="" type="checkbox"/> This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).</p> <p>These annexes consist of a total of 10 sheets.</p>				
<p>3. This report contains indications relating to the following items:</p> <ul style="list-style-type: none"><li>I <input checked="" type="checkbox"/> Basis of the report</li><li>II <input checked="" type="checkbox"/> Priority</li><li>III <input type="checkbox"/> Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</li><li>IV <input checked="" type="checkbox"/> Lack of unity of invention</li><li>V <input checked="" type="checkbox"/> Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement</li><li>VI <input checked="" type="checkbox"/> Certain documents cited</li><li>VII <input checked="" type="checkbox"/> Certain defects in the international application</li><li>VIII <input type="checkbox"/> Certain observations on the international application</li></ul>				
Date of submission of the demand  08/12/1999		Date of completion of this report  04.09.2000		
Name and mailing address of the international preliminary examining authority:   European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 opmu d Fax: +49 89 2399 - 4465		Authorized officer  Döpfer, K-P  Telephone No. +49 89 2399 8547		



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB99/01561

**I. Basis of the report**

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

**Description, pages:**

1-46 as originally filed

**Claims, No.:**

1-58 as received on 31/07/2000 with letter of 28/07/2000

**Drawings, sheets:**

1/8-8/8 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

**II. Priority**

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
- ☐ copy of the earlier application whose priority has been claimed.
  - ☐ translation of the earlier application whose priority has been claimed.
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB99/01561

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

**IV. Lack of unity of invention**

1. In response to the invitation to restrict or pay additional fees the applicant has:

- ☐ restricted the claims.
- ☐ paid additional fees.
- ☐ paid additional fees under protest.
- ☐ neither restricted nor paid additional fees.

2. ☒ This Authority found that the requirement of unity of invention is not complied and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is

- ☐ complied with.
- ☒ not complied with for the following reasons:

see separate sheet

4. Consequently, the following parts of the international application were the subject of international preliminary examination in establishing this report:

- ☒ all parts.
- ☐ the parts relating to claims Nos. .

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/GB99/01561

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement****1. Statement**

Novelty (N)	Yes:	Claims	11-14, 19-21, 26-28, 35-44, 46-48, 55-58
	No:	Claims	1-10, 15-18, 22-25, 29, 30-34, 45, 49-54
Inventive step (IS)	Yes:	Claims	26-28, 46-48
	No:	Claims	1-25, 29-45, 49-58
Industrial applicability (IA)	Yes:	Claims	1-58
	No:	Claims	

**2. Citations and explanations****see separate sheet****VI. Certain documents cited****1. Certain published documents (Rule 70.10)**

and / or

**2. Non-written disclosures (Rule 70.9)****see separate sheet****VII. Certain defects in the international application**

The following defects in the form or contents of the international application have been noted:

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/01561

**Re Item I****Basis of the report****Re Item II****Priority**

1. The priority document in respect of the present application is at present not available to the International Preliminary Examination Authority. In consequence the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document. If it later turns out that this is not correct, the document WO 98 31830 A (BRAX GENOMICS LTD ; THOMPSON ANDREW HUGIN (GB); SCHMIDT GUENTER (GB) 23 July 1998 (1998-07-23) cited in the international search report could become relevant (see also Item VI of this report).

**Re Item III****Non-establishment of opinion with regard to novelty, inventive step and industrial applicability****Re Item IV****Lack of unity of invention**

1. The ISA stated lack of unity of the invention according to Rule 13(1)(2) PCT without inviting the applicant to pay additional fees. The IPEA agrees with the statement of the ISA and considers the following groups of inventions as being present:
  - (i) Method of making a set of labelled compounds and sets of labelled compounds (the labels being cleavable, and the compounds being optionally attached to a particulate solid support) in general (Claims 1-30, 42-43

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/01561

partially)

- (ia) further non-unity  
a specific variable mass-tag system based on tritylamides
  - (ii) Labels, e.g. for oligonucleotide probe analysis, comprising a label attached by a link cleavable to give charged species for analysis by mass spectrometry, e.g. N-hydroxysuccinimide- (NHS-) or amide-bearing monomethoxytrityl systems (Claims 31-41, 42-43 (partially), 44, 55-58)
  - (iii) Inserts for use in laser desorption mass spectrometry, the inserts having target surfaces of glass or of an organic polymer.  
(Claims 45-51)
  - (iv) Analysis of nucleic acids comprising oligonucleotide reagents tagged with moieties suitable for analysis by mass spectrometry (Claims 52-54)
2. The groups of inventions are not linked by a common special technical feature contributing to novelty and inventive step so as to form a single inventive concept underlying the present application (see in particular group (iii) as creating the non-unity *a priori* (without taking the prior art into consideration) (Rule 13(1)(2) PCT). Group (ia) is considered originating from the presence of prior art destroying novelty of the general tagging concept (cf Item V of this written opinion), i.e. non-unity *a posteriori* is to be stated.

**Re Item V****Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Reference is made to the following documents:

D1: WO 95 04160 A (ISIS INNOVATION ;SOUTHERN EDWIN (GB); CUMMINS WILLIAM JONATHAN (GB) 9 February 1995 (1995-02-09) cited in the application

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/01561

- D2: WO 97 27331 A (DARWIN MOLECULAR CORP ;TABONE JOHN C (US); MULLIGAN JOHN T (US); H) 31 July 1997 (1997-07-31)
- D3: EP-A-0 424 819 (MILLIPORE CORP) 2 May 1991 (1991-05-02) & US 5 410 068 A cited in the application
- D4: WO 98 20020 A (KOSTER HUBERT ;LITTLE DANIEL P (US); SEQUENOM INC (US); CANTOR CHA) 14 May 1998 (1998-05-14)
- D5: WO 98 20019 A (REUTER DIRK ;HIGGINS G SCOTT (DE); LOUGH DAVID M (GB); KOESTER HUB) 14 May 1998 (1998-05-14)
- D6: WO 95 28640 A (UNIV COLUMBIA ;COLD SPRING HARBOR LAB (US); STILL W CLARK (US); WI) 26 October 1995 (1995-10-26)
- D7: WO 96 30337 A (SMITHKLINE BEECHAM CORP ;GARIGIPATI RAVI SHANKER (US); ADAMS JERRY) 3 October 1996 (1996-10-03)
- D8: WO 98 31830 A (BRAX GENOMICS LTD ;THOMPSON ANDREW HUGIN (GB); SCHMIDT GUENTER (GB) 23 July 1998 (1998-07-23)
- D9: WO-A-93/06121 (The document was not cited in the international search report. A copy of the document is appended hereto.)
2. The present application relates to the different subject-matters as mentioned under Item IV of this written opinion

**2.1 Novelty (Article 33(2) PCT)**

Subject-matter (i) and (ia): D9 discloses tagging methods for oligomers including the use of cleavable tags (in particular peptides and oligonucleotides). This is considered novelty destroying for the subject-matter of present claims 1-10, 15-18, 22-25, 29 and 30. D9 does not mention explicitly the use of tags which are useful as mass tags. Present claims 11-14, 26-28 can be considered novel in the view of the prior art cited.

Subject-matter (ii): Independent claim 31 pertains to a method of providing a labelled (or tagged ) oligonucleotide or nucleic acid, and removing the label by cleavage which is subjected to MS. D2 (cf e.g. example 15) discloses such a method comprising the steps as claimed. The use of labelled primer molecules is also mentioned. Thus , present claims 31-34 are considered not novel.

None of the prior art documents cited in the ISR discloses subject-matter which



**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/01561

can be regarded as identical with that of present claims 35-41, 42-43 (partially), 44, 55-58.

Subject-matter (iii): D4 and D5 disclose immobilised nucleic acids on solid supports and methods. Present claims 45, 49-51 relate to a product *per se*, i.e. an immobilised compound and its support (designated as insert for MS). The subject-matter of the above mentioned present claims is directed to planar inserts and direct attachment of the immobilised compounds to the support. Direct immobilisation is understood in that the item to be immobilised is attached to the solid support without using linker or spacer molecules which are separately bound to the support prior to the immobilisation of the desired molecule. The disclosure of documents D4 and D5 includes such items. Therefore, novelty cannot be acknowledged for these claims.

The particular embodiments of present claims 46-48 are considered novel in view of the available prior art.

Subject-matter (iv): D1 discloses methods of tagging oligonucleotide molecules with labels and subsequent decoding via MS analysis. Accordingly, the subject-matter of present claims 52-54 is not novel because the system as claimed in said claims must have been used.

## 2.2 Inventive step (Article 33(3) PCT)

The novel subject-matter of present claims 11-14 appears not to involve an inventive step because the contribution of the subject-matter of these claims with respect to the technical teachings of the prior art (e.g. D3-D5) does not lead the skilled person to the conclusion that either a particularly advantageous or surprising solution to a technical problem (i.e. to provide an alternative method for making a set of labelled compounds which can be deciphered, in the present case to introduce mass tags which allow easy deciphering of members of a combinatorial library) has been found.

The novel subject-matter relating to the mass-tags (35-41, 42-43 (partially), 44, 55-58) is considered lacking an inventive step for the following reasons:

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/01561

Mass-tagging for deconvoluting and deciphering sequences is known from the prior art (see documents cited *supra*). Taking D2 as representing the closest prior art, the problem underlying the present application is to be regarded as to provide further labels which are suitable as mass tags. The solution are the claimed substituted monomethoxytrityl compounds. D3 discloses labels with dimethoxytrityl groups which are obviously suitable as mass tags. The skilled person dealing with the problem posed would have come to the claimed solution by combining the teachings of D2 and D3 (combination with D1 would have lead to the same result). The present application discloses the use monomethoxytrityl tags. It is a matter of normal experimentation to modify the degree of methoxy substitution. The presently claimed monomethoxy moieties appear neither to have any particular advantages over the dimethoxy compounds not seem they to exhibit surprising effects which could create presence of inventive step. The particularly designed inserts of claims 46-48 appear to be inventive over the prior art as cited.

Any of the claimed libraries of the application do not involve an inventive step either, because the purpose of a library is in principle to screen for substances possessing a desired property. Since none of the libraries contains structural elements which are considered novel and inventive, it cannot regarded to require inventive activity to create a collection of compounds forming said libraries.

**2.3 Industrial applicability (Article 33(4) PCT)**

The subject-matter of present claims 1-58 appear to comply with the requirements of industrial applicability as stipulated in Article 33(4) PCT.

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/01561

**Re Item VI****Certain documents cited****Certain published documents (Rule 70.10)**

Application No Patent No	Publication date (day/month/year)	Filing date (day/month/year)	Priority date (valid claim) (day/month/year)
WO-A-98/31830	23.07.1998	15.01.1998	15.01.1997
			28.08.1997
			19.12.1997

When entering the European phase the disclosure of the above mentioned document is to be taken into consideration for the assessment of novelty under Article 54(3) EPC.

**Re Item VII****Certain defects in the international application**

1. Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents D2, D4-D9 are not mentioned in the description, nor are these documents identified therein.

PCT/EP99/01561

PC 9103/AM

- 47 -

CLAIMS

- 5 1. A method of making a set of labelled compounds, by the use of a support and a set of labels, which method comprises the steps:
- a) at least one first or intermediate step comprising dividing the support into lots, performing a different chemical reaction on each lot of the support so as either to modify that lot of the support or to couple a chemical moiety to that lot of the support, tagging a fraction of each lot of the support with a different label, and combining the said lots of the support, and
- 10 b) at least one intermediate or final step comprising dividing the support into lots, performing a different chemical reaction on each lot of the support, so as either to modify that lot of the support or to couple a chemical moiety to that lot of the support, tagging a fraction of each lot of the support with a different cleavable label, whereby each different
- 15 cleavable label is linked to a chemical moiety coupled to the support in a different step and forms with that chemical moiety a labelled compound which is separable from the support, and combining the said lots of the support.
- 20
2. The method of claim 1, wherein the support is a particulate solid support.
- 25 3. The method of claim 1 or claim 2, wherein step b) is performed to couple the chemical moiety to a chemical moiety previously coupled to the support.
4. The method of claim 3, wherein the chemical moieties are
- 30 monomer units and the labelled compounds are oligomers.

PCT/EP99/01561

AMENDED SHEET

PCT/GB99/0150

PC 0150/0150

31-07-2000

- 48 -

5. The method of claim 4, wherein the set of labelled compounds is a library of  $n^s$  oligomers, where  $n$  is the number of different monomer units and  $s$  is the number of monomer units in each labelled oligomer, wherein step a) is performed once to couple a different monomer unit to each lot of the support, and step b) is performed  $s-1$  times.
6. The method of claim 5, wherein the set of labelled compounds contains  $n \times s$  different labels.
- 10 7. The method of any one of claims 1 to 6, wherein each labelled compound comprises a single label and at least one chemical moiety.
8. The method of any one of claims 1 to 7, wherein the support is treated to release the said labelled compounds into solution.
- 15 9. The method of any one of claims 1 to 8, wherein from 0.25% to 25% of each lot of the support is tagged in each step with a different label.
- 20 10. The method of any one of claims 1 to 9, wherein the support has cleavable linkers, wherein each cleavable linker has at least one group for chemical synthesis and another group for labelling.
- 25 11. The method of any one of claims 1 to 10, wherein the label is cleaved to give a charged species for mass spectrometry.

PCT/GB99/0150

AMENDED SHEET

RGT/SB99/01361

PC

SIVSAMP

- 49 -

12. The method of any one of claims 1 to 11, wherein each label is a group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.

5

13. The method of claim 12, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary

10

amido, anhydride, carbonyl halide or active ester.

14. The method of any one of claims 1 to 13, wherein the labelled compounds are labelled oligonucleotides.

15

15. A set of labelled compounds wherein a molecule of a compound of the set is tagged with a single cleavable label which identifies the nature and/or the position of a component of that molecule, and different molecules of the same compound are tagged with different labels.

20

16. The set of claim 15, wherein the labelled compounds are releasably attached to a solid support.

17. The set of claim 16, wherein the solid support is particulate.

25

18. The set of claim 15, wherein the labelled compounds are mixed together in solution.

19. The set of any one of claims 15 to 18, wherein the label is cleaved to give a charged species for mass spectrometry.

30

20. The set of any one of claims 15 to 19, wherein each label is a

PAA 011782200

AMENDED SHEET

- 50 -

group of formula  $R^1R^2R^3C$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.

- 5 21. The set of claim 20, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester.

10

22. The set of any one of claims 15 to 21, wherein the labelled compounds are labelled oligonucleotides.

15

23. A library consisting of a plurality of the sets of any one of claims 19 to 22.

20

24. A reagent comprising a solid support which carries on its surface molecules of an oligomer, with different oligomer molecules having the same sequence wherein the oligomer molecules include some shorter oligomer molecules and a shorter oligomer molecule carries a label which identifies the nature and position of a monomer unit of the oligomer molecule.

25

25. The reagent as claimed in claim 24, wherein the solid support is a bead.

30

26. The reagent as claimed in claim 24 or claim 25, wherein the label is joined by a link that is photocleavable to give a charged species for mass spectrometry.

27.

- The reagent of any one of claims 24 to 26, wherein each label

PC 17699/4/56

PC

- 51 -

is a group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.

- 5 28. The reagent of any one of claims 24 to 27, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester.
- 10 29. The reagent of any one of claims 24 to 28, wherein the oligomers are oligonucleotides.
- 15 30. A library consisting of a plurality of the reagents of any one of claims 24 to 29.
- 20 31. A method, which method comprises providing a labelled oligonucleotide or nucleic acid, and removing the label by cleavage to give a charged species which is subjected to matrix-free mass spectrometry.
- 25 32. The method of claim 31, wherein nucleic acid sequencing is performed by the use of a labelled primer and/or a labelled hybridisation probe and/or labelled chain extending nucleotides and/or labelled chain terminating nucleotide analogues, wherein the label is one which is removed by cleavage to give a charged species which is subjected to matrix-free mass spectrometry.



PCT/GB90/0158

PC 0158/90

- 52 -

33. A reagent for an assay in which a labelled probe is partitioned into two fractions one of which is analysed, the probe comprising a ligand joined to a label by a link which is cleavable to give a charged species for matrix-free mass spectrometry.

5

34. The method of claim 33, wherein the ligand is an oligonucleotide.

35. The method of any one of claims 31 to 34, wherein the label  
10 is a group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.

36. The method of claim 35, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$   
15 carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester.

37. A library of probes each comprising a ligand joined to a label  
20 by a link which is cleavable to give a charged species for analysis by mass spectrometry, wherein each different probe has a different label.

38. The library of claim 37, wherein the ligand is an  
25 oligonucleotide.

39. The library of claim 37 or claim 38, wherein each label is a  
30 group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.

PCT/GB90/0158

AMENDED SHEET

767689901581

PC 01/15/2010

- 53 -

40. The library of claim 39, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary  
5 amido, anhydride, carbonyl halide or active ester.

41. A compound of formula  $R^1R^2R^3CY$ , where Y is a leaving group for reaction with a nucleophilic species, and  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group, at  
10 least one of which carries a substituent selected from  $C_1$  -  $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester, provided that  $R^1$ ,  $R^2$  and  $R^3$  together carry at least two amide groups and/or  
15 at least two N-hydroxysuccinimide ester groups.

42. The method of any one of claims 12, 13, 35 and 36, wherein  $R^1R^2R^3C-$  is a substituted monomethoxytrityl group.

20 43. The set of claims 20 or 21, or the reagent of claim 27 or 28, or the library of claims 41 or 42, wherein  $R^1R^2R^3C-$  is a substituted monomethoxytrityl group.

44. The compound of claim 41, wherein  $R^1R^2R^3CY$  is a  
25 substituted monomethoxytrityl compound.

45. An insert for use as a target for laser desorption ionisation mass spectrometry, which insert has a planar target surface of glass or of an organic polymer bearing an immobilised compound for analysis, directly  
30 attached to said target surface.

Amended 23.03.2010

AMENDED SHEET

PC 1/0000/01/001

PC 1/0000/01/001

- 54 -

46. The insert of claim 45, wherein the compound to be analysed comprises a group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.

5

47. The insert of claim 46, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_{1-20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester.

10

48. The insert of claim 46 or 47, wherein  $R^1R^2R^3C-$  is a substituted monomethoxytrityl group.

15

49. The insert of any one of claims 45 to 48, wherein the target surface carries an array of immobilised compounds for analysis.

20

50. The insert of anyone of claims 45 to 49, wherein compounds are immobilised on target surfaces of glass by means of epoxysilane chemistry or isothiocyanate chemistry or mercaptosilane chemistry or polylysine.

25

51. A kit comprising a mass spectrometer and a supply of inserts bearing an immobilised compound for analysis directly attached to said insert surface, for use as targets for matrix-free laser desorption mass spectrometry, having target surfaces of glass or of an organic polymer.

PC 1/0000/01/001

AMENDED SHEET

PC-PCB00/01551

PC

- 55 -

52. A system for analysing nucleic acids comprising:

- a solid support carrying an array of nucleic acids to act as targets for analysis or as probes to capture a target;
- oligonucleotide reagents tagged with moieties suitable for analysis by mass spectrometry;
- reagents and apparatus for biochemical procedures to allow specific interaction between the tagged oligonucleotides and the target;
- a means to introduce the samples into a mass spectrometer;
- a mass spectrometer.

10

53. A system for analysing nucleic acids on a solid support comprising:

- a solid support carrying an array of nucleic acids to act as targets for analysis or as probes to capture a target;
- oligonucleotide reagents, tagged with moieties suitable for analysis by mass spectrometry;
- reagents and apparatus for biochemical procedures to allow specific interaction between the tagged oligonucleotides and the target carried out on the solid support surface;
- a means to introduce the solid support into a mass spectrometer;
- a mass spectrometer.

15

20

54. An automated system for analysing nucleic acids comprising:

- oligonucleotide reagents, tagged with moieties suitable for analysis by mass spectrometry;
- a mass spectrometer;
- a computer to carry out the analysis;
- software to interpret a mass spectrum.

25

30

PC/UG/09/01501

PC/SEM/STAND

- 56 -

55. A nucleotide or oligonucleotide labelled with a tag suitable for analysis by mass spectrometry, said labelled nucleotide or oligonucleotide being suitable for enzymatic incorporation, wherein the tag is a compound of formula  $R^1R^2R^3CY$ , where Y is a leaving group for reaction with a nucleophilic species, and  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group, at least one of which carries a substituent selected from  $C_1 - C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester.

56. The method of any one of claims 31 to 36, wherein 4s different labels are used, where the labelled oligonucleotide or nucleic acid contains s bases and each label is indicative of the position and identity of a nucleotide residue of the labelled oligonucleotide or nucleic acid.

57. The method of claim 56, wherein for a base position of the labelled oligonucleotide or nucleic acid, four regions of a mass spectrum corresponding to the masses of the four possible labels (including their isotopic variants) are examined and compared with an expected mass spectrum of the label.

58. The method of any one of claims 31 to 36, wherein each possible oligonucleotide or nucleic acid containing s bases is compared in turn against a mass spectrum comprising the s different tag regions, to identify the oligonucleotide having the best fit.



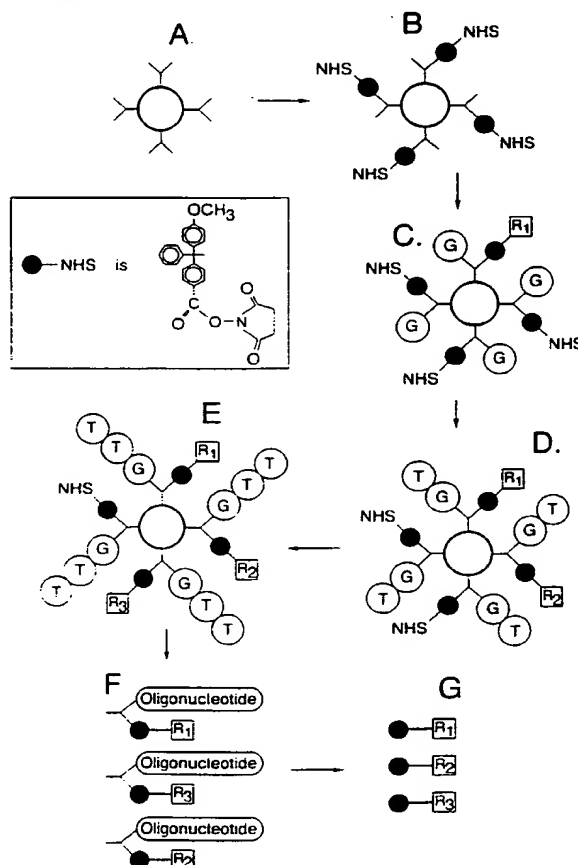
## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification <sup>6</sup> : <b>C07H 21/00, C07B 61/00</b>		A3	(11) International Publication Number: <b>WO 99/60007</b>
			(43) International Publication Date: 25 November 1999 (25.11.99)
(21) International Application Number: PCT/GB99/01561		(74) Agents: PENNANT, Pyers et al.; Stevens Hewlett & Perkins, 1 Serjeants' Inn, Fleet Street, London, Greater London EC4Y 1NT (GB).	
(22) International Filing Date: 17 May 1999 (17.05.99)			
(30) Priority Data: 98303873.8 15 May 1998 (15.05.98) EP		(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): ISIS INNOVATION LIMITED [GB/GB]; 2 South Parks Road, Oxford, Oxfordshire OX1 3UB (GB).			
(72) Inventors; and			
(75) Inventors/Applicants (for US only): SOUTHERN, Edwin, Mellor [GB/GB]; 12 School Road, Kidlington, Oxford, Oxfordshire OX5 2HB (GB). SHCHEPINOV, Mikhail, Sergeevich [RU/GB]; 8 Barns Hay, Old Marston, Oxford, Oxfordshire OX3 0PN (GB). HOUSBY, John, Nicholas [GB/GB]; Gazebo Cottage, High Street, Hook Norton, Banbury, Oxfordshire OX15 5NQ (GB). HAMILTON, Alan, Lewis [GB/GB]; Ashdown, 15 Church Grove, Little Chalfont, Buckinghamshire HP6 6SH (GB). ELDER, John, Kenneth [GB/GB]; 14 St Mary's Close, Kidlington, Oxford, Oxfordshire OX5 2AY (GB).		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
		(88) Date of publication of the international search report: 20 January 2000 (20.01.00)	

(54) Title: LIBRARIES OF OLIGOMERS LABELLED WITH DIFFERENT TAGS

## (57) Abstract

A method of making a set of labelled compounds by the use of a preferably particulate support, comprises dividing the support into lots, performing a different chemical reaction on each lot of the support, e.g. to couple a chemical moiety to that lot of the support, tagging a fraction of each lot of the support with a different label, and combining the said lots of the support. The steps are repeated several times, preferably to build up oligomer molecules carrying labels which identify the nature and position of a monomer unit of the oligomer, and which are releasable from the support. Preferred labels, which are releasable from the compounds by cleavage to provide charged groups for analysis by mass spectrometry, are groups of the trityl (trimethylphenyl) family. Also claimed are libraries of these labels and their use in assays and nucleic acid analysis methods.



**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/01561

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C07H21/00 C07B61/00

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C07B C07H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 04160 A (ISIS INNOVATION ;SOUTHERN EDWIN (GB); CUMMINS WILLIAM JONATHAN (GB) 9 February 1995 (1995-02-09) cited in the application page 47; claims 1-15; figure 3	52-54
Y		31-44, 55-58
A		1-30
Y	WO 97 27331 A (DARWIN MOLECULAR CORP ;TABONE JOHN C (US); MULLIGAN JOHN T (US); H) 31 July 1997 (1997-07-31) page 12 -page 16 page 28 -page 29; figures 9,10; example 16 -/--	31-44, 55-58

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

### \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

15 November 1999

Date of mailing of the international search report

09/12/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

Hart-Davis, J



# INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/01561

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 424 819 A (MILLIPORE CORP) 2 May 1991 (1991-05-02) page 8 page 10 figure 1 & US 5 410 068 A cited in the application ---	31-44, 55-58
X	WO 98 20020 A (KOSTER HUBERT ;LITTLE - DANIEL P (US); SEQUENOM INC (US); CANTOR CHA) 14 May 1998 (1998-05-14) claims 1,20-24,83,89,90,100-102 ---	45,49-51
X	WO 98 20019 A (REUTER DIRK ;HIGGINS G SCOTT (DE); LOUGH DAVID M (GB); KOESTER HUB) 14 May 1998 (1998-05-14) page 19, line 5 -page 21, line 23; figures 10-13 ---	45,49-51
A	WO 95 28640 A (UNIV COLUMBIA ;COLD SPRING HARBOR LAB (US); STILL W CLARK (US); WI) 26 October 1995 (1995-10-26) page 5 -page 6 page 31 -page 32 page 51 -page 57 ---	1-30
A	WO 96 30337 A (SMITHKLINE BEECHAM CORP ;GARIGIPATI RAVI SHANKER (US); ADAMS JERRY) 3 October 1996 (1996-10-03) the whole document ---	31-44, 55-58
P,Y	WO 98 31830 A (BRAX GENOMICS LTD ;THOMPSON ANDREW HUGIN (GB); SCHMIDT GUENTER (GB) 23 July 1998 (1998-07-23) claims 1-50; figures 7,8 -----	31-44, 55-58
A		1-30

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/ 01561

### Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:  
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:  
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

### Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☒ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

#### Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: 1-30, 42-43 (partially)

A set of labelled compounds (optionally attached to a particulate solid support) wherein different molecules / lots of the set are labelled with different tags.

2. Claims: 31-41, 42-43 (partially), 44, 55-58

Labels, e.g. for oligonucleotide probe analysis, comprising a label attached by a link cleavable to give a charged species for analysis by mass spectrometry, e.g. N-hydroxysuccinimide- (NHS-) or amide-bearing monomethoxytrityl systems.

3. Claims: 45-51

Inserts for use in laser desorption mass spectrometry, the inserts having target surfaces of glass or of an organic polymer.

4. Claims: 52-54

Analysis of nucleic acids comprising oligonucleotide reagents tagged with moieties suitable for analysis by mass spectrometry.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/01561

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9504160 A	09-02-1995	AT 159767 T AU 695349 B AU 7269194 A CA 2168010 A CN 1131440 A DE 69406544 D DE 69406544 T DK 711362 T EP 0711362 A EP 0778280 A ES 2108479 T FI 960403 A HU 73802 A JP 9501830 T NO 960370 A US 5770367 A	15-11-1997 13-08-1998 28-02-1995 09-02-1995 18-09-1996 04-12-1997 26-02-1998 22-12-1997 15-05-1996 11-06-1997 16-12-1997 29-01-1996 30-09-1996 25-02-1997 28-03-1996 23-06-1998
WO 9727331 A	31-07-1997	AU 2247397 A CA 2243560 A CN 1212021 A CZ 9802184 A EP 0868535 A HU 9901321 A NZ 331044 A PL 328271 A	20-08-1997 31-07-1997 24-03-1999 16-12-1998 07-10-1998 28-07-1999 25-02-1999 18-01-1999
EP 0424819 A	02-05-1991	US 5410068 A DE 69015566 D DE 69015566 T JP 3279371 A	25-04-1995 09-02-1995 10-08-1995 10-12-1991
WO 9820020 A	14-05-1998	AU 5106998 A AU 5198098 A EP 0954612 A EP 0937096 A NO 992168 A NO 992169 A WO 9820166 A AU 5247298 A DE 19782097 T EP 0937097 A NO 992167 A WO 9820019 A	29-05-1998 29-05-1998 10-11-1999 25-08-1999 06-07-1999 06-07-1999 14-05-1998 29-05-1998 14-10-1999 25-08-1999 05-07-1999 14-05-1998
WO 9820019 A	14-05-1998	US 5900481 A AU 5106998 A AU 5247298 A DE 19782097 T EP 0954612 A EP 0937097 A NO 992167 A NO 992168 A WO 9820166 A AU 5198098 A EP 0937096 A NO 992169 A WO 9820020 A	04-05-1999 29-05-1998 29-05-1998 14-10-1999 10-11-1999 25-08-1999 05-07-1999 06-07-1999 14-05-1998 29-05-1998 25-08-1999 06-07-1999 14-05-1998

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/01561

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9528640 A	26-10-1995	US 5565324 A AU 2292695 A CA 2187792 A CN 1151793 A EP 0755514 A HU 74985 A JP 10502614 T NO 964332 A US 5968736 A US 5789172 A	15-10-1996 10-11-1995 26-10-1995 11-06-1997 29-01-1997 28-03-1997 10-03-1998 03-12-1996 19-10-1999 04-08-1998
WO 9630337 A	03-10-1996	EP 0817771 A JP 11502854 T	14-01-1998 09-03-1999
WO 9831830 A	23-07-1998	AU 5570098 A AU 1770499 A WO 9932501 A	07-08-1998 12-07-1999 01-07-1999



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>6</sup> :</b> <b>C07H 21/00</b>	<b>A2</b>	<b>(11) International Publication Number:</b> <b>WO 99/60007</b> <b>(43) International Publication Date:</b> 25 November 1999 (25.11.99)
<b>(21) International Application Number:</b> PCT/GB99/01561 <b>(22) International Filing Date:</b> 17 May 1999 (17.05.99)  <b>(30) Priority Data:</b> 98303873.8      15 May 1998 (15.05.98)      EP  <b>(71) Applicant (for all designated States except US):</b> ISIS INNOVATION LIMITED [GB/GB]; 2 South Parks Road, Oxford, Oxfordshire OX1 3UB (GB).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SOUTHERN, Edwin, Mellor [GB/GB]; 12 School Road, Kidlington, Oxford, Oxfordshire OX5 2HR (GB); SHCHEPINOV, Mikhail, Sergeevich [RU/GB]; 8 Bains Hay, Old Marston, Oxford, Oxfordshire OX3 0PN (GB); HOUSBY, John, Nicholas [GB/GB]; Gazebo Cottage, High Street, Hook Norton, Banbury, Oxfordshire OX15 5NQ (GB). HAMILTON, Alan, Lewis [GB/GB]; Ashdown, 15 Church Grove, Little Chalfont, Buckinghamshire HP6 6SH (GB). ELDER, John, Kenneth [GB/GB]; 14 St Mary's Close, Kidlington, Oxford, Oxfordshire OX5 2AY (GB).		<b>(74) Agents:</b> PENNANT, Pyers et al.; Stevens Hewlett & Perkins, 1 Serjeants' Inn, Fleet Street, London, Greater London EC4Y 1NT (GB).  <b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> REAGENT AND METHOD  <b>(57) Abstract</b> <p>A method of making a set of labelled compounds by the use of a preferably particulate support, comprises dividing the support into lots, performing a different chemical reaction on each lot of the support, e.g. to couple a chemical moiety to that lot of the support, tagging a fraction of each lot of the support with a different label, and combining the said lots of the support. The steps are repeated several times, preferably to build up oligomer molecules carrying labels which identify the nature and position of a monomer unit of the oligomer, and which are releasable from the support. Preferred labels, which are releasable from the compounds by cleavage to provide charged groups for analysis by mass spectrometry, are groups of the trityl (trimethylphenyl) family. Also claimed are libraries of these labels and their use in assays and nucleic acid analysis methods.</p>		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon			PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

## REAGENT AND METHOD

5                    This invention concerns reagents of the kind which comprise  
a product which is built up using stepwise reactions, often chemical  
reactions, and associated tag moieties which track the synthetic pathway  
and/or the reagents used. The product will often be an oligomer and the  
tags define the identity and position of at least one monomer residue in the  
10   oligomer. Such reagents are useful in assay methods in which they can  
generate much more information than can be generated by a simple  
labelled analyte. Sets and libraries of such reagents can be created by  
combinatorial chemistry and are valuable for screening large numbers of  
compound e.g. for biological activity. In preferred systems according to the  
15   invention, positively charged tag groups are generated for analysis by mass  
spectrometry by cleavage, e.g. photocleavage of neutral molecules.

WO 95/04160 describes a reagent which comprises:

- a)                    an analyte moiety comprising at least two analyte residues,  
and linked to
- 20   b)                    a tag moiety comprising one or more reporter groups adapted  
for detection by mass spectrometry, wherein a reporter group designates  
an analyte residue, and the reporter group at each position of the tag  
moiety is chosen to designate an analyte residue at a defined position of  
the analyte moiety. A plurality of such reagents, each comprising a  
25   different analyte moiety, provides a library of reagents which may be used  
in assay methods involving a target substance. Analysis of the tag  
moieties indicates the nature of the analyte moiety bound to the target  
substance.

WO 94/08051 describes a system used to make  
30   simultaneously a library of all oligomers each attached to a bead. Any  
individual bead made by a split and mix process carries a unique chemical



product, and this is true of each bead which goes through the same synthetic pathway. Two coupling steps are used at each point in the process: one step affects the synthon or ligand; the other alters the structure of a tag which is also carried on the bead. Tags are designed to  
5 identify the steps through which the bead has been taken.

It is an object of this invention to provide a set or library of labelled compounds which may be synthesised on a support and may be used either attached to or separated from that support.

In one aspect the invention provides a method of making a  
10 set of labelled compounds, by the use of a support and a set of labels, which method comprises the steps:

- a) at least one first or intermediate step comprising dividing the support into lots, performing a different chemical reaction on each lot of the support so as either to modify that lot of the support or to couple a chemical  
15 moiety to that lot of the support, tagging a fraction of each lot of the support with a different label, and combining the said lots of the support, and
- b) at least one intermediate or final step comprising dividing the support into lots, performing a different chemical reaction on each lot of the support, so as either to modify that lot of the support or to couple a  
20 chemical moiety to that lot of the support, tagging a fraction of each lot of the support with a different label, whereby each different label is linked to a chemical moiety coupled to the support in a different step and forms with that chemical moiety a labelled compound which is separable from the support, and combining the said lots of the support.

25 The method uses a support which is repeatedly divided into lots which are then recombined. The support may be a massive support e.g. a flat sheet or silicon chip or microtitre plate which is divided e.g. by masking into regions for performing the different chemical reactions. The support may be a polymeric material which is soluble in some solvents and  
30 not in others, and which is separated into lots or recombined e.g. by precipitation or dissolution. Most usually the support will be particulate, e.g.

pins or fibres or capillaries or preferably beads. Derivatised beads for performing combinatorial chemistry by a split and mix strategy are commercially available and can be used here. A preferred particulate support comprises beads having cleavable linkers, wherein each cleavable  
5 linker has one group for defined chemical procedures e.g. oligomer synthesis and another group for labelling. By this means it is possible at the end of the synthesis, to recover the labelled chemical products e.g. oligomers into solution.

The method of the invention involves performing at least one  
10 step a) and at least one step b), most usually at least three steps in all. Each step involves performing a reaction, generally but not necessarily a chemical reaction. An example of such a reaction might be the removal of a protective group so as to leave a primary amine or hydroxyl or carboxylic acid group. Most usually, the chemical reaction involves coupling a  
15 chemical moiety to the support. The chemical moiety will usually be an organic chemical group, for example as described in WO 94/08051. While successive chemical moieties may be attached to the support through separate linkers, more usually, successive chemical moieties will be joined to each other to form a chain extending from the support. Preferably the  
20 chemical moieties are monomer units which are built up to form oligomer chains.

In a preferred method according to the invention, the set of labelled compounds is a library of  $n^s$  labelled oligomers, where  $n$  is the number of different monomer units and  $s$  is the number of monomer units in  
25 each labelled oligomer, wherein step a) is performed once to couple a different monomer unit to each lot of the support, and step b) is performed  $s-1$  times.

The oligomer may be for example an oligonucleotide or an oligopeptide. When the oligomer is an oligonucleotide or analogue, then  $n$   
30 is generally 4. When the oligomer is an oligopeptide, then  $n$  is generally about 20 when only natural amino acids are used. But the principles of the

invention are equally applicable to other oligomers formed from other polymerisable monomers. The value of  $s$  is not critical, and may typically be from 2 – 100 e.g. 3 – 20 or more.

The fraction of each lot that is tagged in each step is  
5 generally less than 50%. Preferably from 0.25% to 25% of each lot of the support is tagged in each step with a different label. Preferably the support has cleavable linkers, wherein each cleavable linker has at least one group for chemical reaction e.g. chemical synthesis and another group for labelling. Preferably each resulting labelled compound comprises a single  
10 label and at least one chemical moiety.

The method involves the use of a set of up to and including  $n \times s$  different labels. Although the nature of the labels is not critical, it is a preferred feature of the invention that each different label be distinguishable by the analytical procedure used to detect the labels.  
15 Groups used as labels should be much more stable to acidic (or other chemical) treatment involved in oligomer synthesis compared to the protecting groups commonly used (e.g. DMT groups to provide 5' or 3'-protection in nucleotide synthons). Preferred labels are those in which a charged group, preferably a positively charged group is generated by  
20 cleavage e.g. photocleavage of a neutral molecule for analysis by mass spectrometry. Examples of such preferred labels are discussed below.

In a preferred embodiment, a split and mix strategy requires a solid support carrying cleavable linkers with three arms – one to attach to the solid support through a cleavable bond; one to initiate synthesis of a  
25 chemical product e.g. oligomer; and a third for attachment of the tags. The sites for coupling of synthon and tag monomers will optionally be protected by removable groups. The process can be illustrated by the synthesis of oligomers on a particulate solid support.

At each stage in the synthetic route, the particles of the  
30 support are first combined and mixed, and then divided into  $n$  lots, where  $n$  is the number of different monomers – 4 in the case of natural nucleotides

– and each monomer is coupled to its site on one lot of the support. A unique tag representing the monomer just added and its position in the sequence is then coupled to a fraction of the support, corresponding approximately to the number of monomers in the final oligomer (i.e.  $1/s$  for an oligomer with  $s$  monomer units). Alternatively, a tag may be coupled to a fraction of the support before or simultaneous with, rather than after, the monomer which it represents. Partial coupling may be achieved in a number of different ways. For example, (i) a protecting group on the site may be partially removed; (ii) the coupling may be taken to a fraction of completion; (iii) a fraction of the support may be removed and coupling taken to completion before the fraction is returned to the pool. As the coupling steps proceed, the oligomer is extended one unit at a time, and the tags are added one at a time. The end result is a mixture of molecules on each particle; each molecule will carry the same sequence of monomers in the oligomer, but a fraction,  $1/s$  for  $s$ -mers, will carry the tag added at any of the  $s$  coupling steps.

An example of this embodiment is shown in Figure 1 of the accompanying drawings. At A, is illustrated a solid support in the form of a bead derivatised with cleavable linkers each having two arms. At B, one of the two arms of each linker has been reacted with a trityl group carrying a succinimidyl substituent. At C, the other branch of each linker has been reacted with a nucleotide residue shown as G; and one portion of the NHS groups has been substituted by a label  $R_1$ . At D, oligonucleotide synthesis has continued by formation of dimer chains GT; and a second portion of the NHS groups has been substituted by a second label  $R_2$ . At E, oligonucleotide synthesis has continued by formation of chains GTT; and a third portion of the NHS groups has been substituted by a label  $R_3$ . At F, ammonolysis of the beads has given rise to a pool of oligonucleotides of the same sequence, in which each one is attached to a different tag. At G, photolysis has detached three derivatised trityl groups for analysis by mass spectrometry. The split and mix approach ensures that all the

oligonucleotides attached to any bead have the same s-mer sequence; and that the bead also carries a total of s different labels, each of which indicates the position and identity of one monomer residue of the oligomer.

An alternative way of partial coupling is to cap the extension  
5 of a fraction of the chemical compounds e.g. oligomers with a stable tag group at each extension step. For example, in the case of oligonucleotide synthesis, the coupling agents could include a small proportion of a phosphoramidite protected by one of the stable trityl groups described below as mass tags. Elongation will produce a major proportion with the  
10 desired base and a small fraction with a corresponding tag marking the nature and position of the base.

An example of this embodiment is illustrated in Figure 2 of the accompanying drawings. Oligonucleotide synthesis is performed on derivatised beads A, the first, second and third stages of this synthesis  
15 being shown as B, C and D. Each of four phosphoramidite reagents contains a small fraction depending on the length of the oligomer, preferably less than 1/s, of a capping phosphoramidite bearing a very acid-stable NHS-substituted trityl group. After each stage of synthesis, all incorporated NHS groups are reacted with an amine thereby attaching a  
20 label. For synthesis of longer oligonucleotides o-methyl phosphoramidite could be used to withstand repetitive amination reactions. The three different labels used in B, C and D are shown in Figure 2 as R<sub>1</sub>, R<sub>2</sub> and R<sub>3</sub>. At the end of synthesis, the oligonucleotides are deprotected by treatment with ammonia, but remain attached to the beads. Thus each bead carries  
25 a plurality of s-mer oligomers of identical sequence, together with a total of s different substituted trityl labels each of which indicates the identity and position of a monomer unit of the oligomer. The beads are used in an assay procedure. Thereafter photolysis of a bead generates charged substituted trityl moieties E for detection by mass spectroscopy.  
30 Alternatively the labelled oligonucleotides can be released into solution.

In another aspect, this invention provides a set of labelled

compounds wherein a molecule of a compound of the set is tagged with a single label which identifies the nature and/or the position of a component of that molecule, and different molecules of the same compound are tagged with different labels. The set of labelled compounds may be  
5 releasably attached to a solid support e.g. beads; or may be mixed together in solution.

Also envisaged according to the invention is a library consisting of a plurality of the sets of the labelled compounds as herein defined, e.g. a library of  $n^s$  labelled oligomers, where  $n$  is the number of  
10 different monomer units and  $s$  is the number of monomer units in each labelled oligomer.

In another aspect (e.g. as illustrated in Figure 2) the invention provides a reagent comprising a solid support which carries on its surface molecules of an oligomer, with different oligomer molecules having the  
15 same sequence wherein the oligomer molecules include some shorter oligomer molecules and a shorter oligomer molecule carries a label which identifies the nature and position of a monomer unit of the oligomer molecule. A library consists of a plurality of the said reagents, in which the solid supports are preferably beads.

20 Preferred features of the labels used herein are:

- They should be attached by linkages which are stable to the chemical procedures used in the preparative method and those used to detach the resulting chemical compound e.g. oligomer from a solid support. The trityl residues described below are stable throughout the procedures  
25 used to synthesise oligonucleotides.
- They should have properties which allow up to  $n \times s$  labels to be distinguished by the analytical procedure used to detect them, as each chemical moiety or reaction is tagged uniquely. In an example below, it is shown how all 262144 nonanucleotides can be coded uniquely using 36  
30 different tag monomers. This number is readily achieved using the trityl derivatives described below. Alternatively, but less preferably, the same

number of 9-mers could be coded for by 18 binary tags or even by a unique combination of 9 tags as described in WO 94/08051.

- On cleavage, e.g. by photocleavage, chemical cleavage using acidic conditions, or enzymatic methods, from the parent molecule, they should generate stable species, either neutral molecules or preferably charged ions, for analysis by mass spectrometry. Mass spectrometry is a preferred method of analysis, allowing for the simultaneous detection of hundreds of labels. This property, of generating a preferably charged group by photocleavage of a neutral molecule, ensures that the ions are brought into the vapour phase without the need for added matrix. Therefore it is not necessary to search for "hot spots" as is the case when matrix is added. Not having matrix present also allows for further biochemical processes e.g. oligonucleotide ligation. In certain cleavage methods such as those involving acid, the addition of matrix may enhance the sensitivity of detection.

Bearing in mind these criteria, preferred labels according to the invention are groups of the formula  $R^1R^2R^3C-$  where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted. These are groups of the trityl (triphenylmethyl) family. Other possible labels include troponium and those discussed in WO 97/27331. Trityl groups have the desirable property that they are readily cleaved by illumination with a laser in a mass spectrometer. Sensitivity of detection of trityl groups is high because of the stability of the positively charged carbonium ion. This sensitivity gives rise to a number of advantages, e.g. there are enough trityl groups in a molecular monolayer such as results if trityl labelled molecules are tethered covalently to a surface.

Preferably at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido,

anhydride, carbonyl halide or active ester. Hydrogen atoms in these substituents may be partly or wholly replaced by deuterium or halogen e.g. fluorine; this improves the range available for analysis by mass spectrometry.

5                    Preferably each of  $R^1$ ,  $R^2$  and  $R^3$  is aryl, more preferably phenyl. While substituents may be present at any point in the aromatic (e.g. phenyl) ring, para-substituents are convenient and are preferred. The substituents may be present to confer desired physical or chemical properties on the trityl (or other) group. For example, electron withdrawing  
10 groups at ortho or para positions increase the stability of trityl groups to acid hydrolysis. Substituents may be present to alter the formula weight of the trityl (or other) group, so as to enable easy detection and discrimination by mass spectrometry. Non-radioactive isotopic substituents are suitable for this purpose, e.g. small alkyl groups containing 1, 2 or 3 deuterium  
15 atoms. Preferred substituents are amine or amide groups. There is a considerable number of amines having different molecular weights that are commercially available and that can be used to provide substituted trityl groups having distinctive formula weights, see for example Table 1 below.

                    The masses of the majority of commercially available amines  
20 lie in the range of 50 – 250 Da. For some applications it would be desirable to have up to a few hundred mass-tags. The resolution of the tags in TOF-mass spectrometry was found to be satisfactory with at least 4 Da difference between the masses of tags. Therefore, the above range of amines can only yield about 50 different tags. To increase this amount  
25 using the same pool of amines, it is possible to incorporate two or four or even more amide substituents per trityl group, and this is illustrated in the experimental section below.

                    The principle of the system is illustrated in Figure 3 of the accompanying drawings. At A, an oligonucleotide has been synthesised on  
30 a CPG support. At B, a 5'-hydroxyl group of the oligonucleotide has been replaced by an NHS-substituted trityl group. At C, an amide group NHR



has been introduced, in which R is chosen to have a characteristic formula weight. At D, the labelled oligonucleotide has been released into solution for use in an assay procedure. At E, the NHR-substituted trityl group has been volatilised by photolysis and has been detected by mass  
5 spectrometry.

The above mass spectrometry labels are useful in a variety of other biochemical methods and manipulations. Thus according to another aspect, the invention provides a nucleic acid determination method, which method comprises providing a labelled oligonucleotide or nucleic acid, and  
10 removing the label by cleavage to give a charged species which is subjected to mass spectrometry. Preferably nucleic acid analysis, e.g. sequencing or sequence difference analysis, is performed by the use of a labelled primer and/or labelled chain extending nucleotides and/or labelled chain terminating nucleotide analogues, wherein the label is as described  
15 above.

In another aspect, the invention provides an assay method in which a labelled probe is partitioned into two fractions of which one is determined, the probe comprising a ligand joined to a label by a link which is cleavable to give a charged species for analysis by mass spectrometry.  
20 The invention also includes a library of probes, each comprising a ligand joined to a label by a link which is cleavable to give a charged species for analysis by mass spectrometry, wherein each different probe has a different label. Preferably the labels are as described above.

Certain of the labels are envisaged as new compounds *per se* according to the invention. These are compounds of the formula  
25  $R^1R^2R^3CY$ ; where Y is a leaving group e.g. halide or tosylate for displacement by a nucleophile e.g. a thiol, alcohol or amine group; and  $R^1$ ,  $R^2$  and  $R^3$  are as defined above, with the proviso that  $R^1$ ,  $R^2$  and  $R^3$  together carry at least two amide groups and/or at least two reactive groups  
30 for coupling e.g. N-hydroxysuccinimide ester groups.

In addition, the inventors have manufactured a disposable

glass insert for use as a target surface for laser desorption ionisation mass spectrometry. The glass target may be used for analysis of samples spotted and dried directly on to the glass surface. The glass target may also be chemically activated and used as a solid support for immobilised  
5 nucleic acids or other compounds using methods already developed. Complementary nucleic acids, mass-tagged oligonucleotides or other compounds isolated and localised on the glass target may then be subjected to direct analysis by laser desorption ionisation mass spectrometry. One advantage of using a solid support is that it may be  
10 introduced directly into a mass spectrometer for subsequent detection and avoids unnecessary liquid handling of the sample. Organic polymeric surfaces such as polypropylene are possible alternatives to glass.

Any surface chemistry developed for attachment of compounds to glass may be used to immobilise these compounds directly  
15 on to a target for laser desorption ionisation mass spectrometry or matrix-assisted laser desorption ionisation mass spectrometry. For example 3-mercaptopropyl silane derivatisation (Rogers, *et al* 1999) or amine derivatisation (Beattie, *et al* 1995; Chen, *et al* 1999) for the attachment of nucleic acids. The glass inserts are significantly cheaper than conventional  
20 inserts and are truly disposable. Mass spectrometry performance is unaffected. (See Example 4 below).

The invention also provides an insert for use as a target for laser desorption ionisation mass spectrometry, which insert has a target surface of glass carrying an immobilised compound for analysis.

25 The invention also provides a kit comprising a mass spectrometer and a supply of inserts, for use as targets for laser desorption mass spectrometry, having target surfaces of glass.

In a preferred embodiment of the invention, a system for analysing nucleic acids comprises:

- 30 • a solid support carrying an array of nucleic acids to act as targets for analysis or as probes to capture a target;

- oligonucleotide reagents tagged with moieties suitable for analysis by mass spectrometry;
- reagents and apparatus for biochemical procedures to allow specific interaction between the tagged oligonucleotides and the target;
- 5 • a means to introduce the samples into a mass spectrometer;
- a mass spectrometer.

In a more preferred embodiment of the invention, a system for analysing nucleic acids on a solid support comprises:

- a solid support carrying an array of nucleic acids to act as  
10 targets for analysis or as probes to capture a target;
- oligonucleotide reagents, tagged with moieties suitable for analysis by mass spectrometry;
- reagents and apparatus for biochemical procedures to allow specific interaction between the tagged oligonucleotides and the target  
15 carried out on the solid support surface;
- a means to introduce the solid support into a mass spectrometer;
- a mass spectrometer.

In a further preferred embodiment of the invention, an  
20 automated system for analysing nucleic acids comprises:

- oligonucleotide reagents, tagged with moieties suitable for analysis by mass spectrometry;
- a mass spectrometer;
- a computer to carry out the analysis;
- 25 • software to interpret a mass spectrum.

Computer programs are provided for oligonucleotide sequence determination by mass spectrometry.

Each base and base position in an oligonucleotide is associated with a unique mass tag.

- 30 For oligonucleotides of length  $s$ ,  $4s$  tags are needed to distinguish between all  $4^s$  possible oligonucleotides.

Careful choice of tags ensures that all tags have sufficiently different masses to avoid ambiguity in tag assignment when analysing a mass spectrum.

The chemical formula for each tag is known, so each tag's  
5 monoisotopic mass can be calculated.

The isotopic abundances of the elements in the tag are also known, so a complete distribution of masses and abundances of all isotopic variants of each tag can be calculated.

For the tags used so far, the major heavy isotopes of a tag  
10 are those due to the presence of  $^{13}\text{C}$ , and a typical isotopic abundance distribution is that for  $\text{C}_{27}\text{H}_{30}\text{O}_2\text{N}$ , with relative abundances of 73:22:3 for isotopic masses 400.228, 401.231 and 402.234 respectively. These abundance distributions characterise the tags' presence in a mass spectrum, and help to distinguish tags from other features in the spectrum.

15 The use of elements such as chlorine or bromine in mass tags further aids tag detection and identification, since these elements have markedly different isotopic abundances from that of carbon:

for  $^{35}\text{Cl}$ : $^{37}\text{Cl}$  the abundance ratio is 76:24

and for  $^{79}\text{Br}$ : $^{81}\text{Br}$  it is 51:49.

20 Mass tags containing these elements will therefore have their own characteristic isotopic distributions. In general, the aim is to design tags with characteristic sets of masses which facilitate identification amongst a background of chemical 'noise'.

Mass standards are included in each spectrum to allow the  
25 spectrum's mass axis to be calibrated. Use of mass standards both within and on either side of the tag mass range ensures accurate mass measurement throughout this range. Ions representing a complete set of possible masses are often seen in mass spectra and these represent the ideal calibration set.

30 A program has been written to calculate the isotopic abundance distribution and corresponding isotopic masses of any mass

tag, using the known masses and isotopic abundances of the elements in each tag. This information is calculated for all mass tags available for use in tagging oligonucleotides.

A second program uses this information to determine the  
5 presence of mass tags and hence the sequence in the mass spectrum generated by an oligonucleotide, and works as follows. For each base position in the oligonucleotide, the four regions of the mass spectrum corresponding to the masses of the four possible tags (including their isotopic variants) are examined and compared with the expected tag  
10 spectrum. The comparison is done either by identification of spectral peak positions and amplitudes and their differences from those of the potential tag, or by measuring the sum of squares of residuals between the experimental spectrum and that expected from the potential tag. In either case, the four potential tags are ranked by the chosen measure and the  
15 best tag is used to assign a base to that base position.

A more powerful approach is to examine each possible oligonucleotide in turn, obtaining a goodness of fit over all s tag regions by the method described above, and then ranking the oligonucleotide sequences by this measure.

20 Reference is directed to the accompanying drawings in which:-

Figure 1 is a diagram of a coding strategy using trityl-based mass-tags which are attached to oligonucleotides in solution.

Figure 2 is a diagram of a coding strategy using trityl-based  
25 mass tags, with mass tags and oligonucleotides attached to beads.

Figure 3 is a diagram showing the synthesis and detection of an MMT-tagged oligonucleotide.

Figure 4 shows synthesis of various trityl-based mass-tags.

Figures 5 and 6 are diagrams showing a PCR and  
30 immobilisation strategy followed by hybridisation and ligation.

Figures 7 and 8 are mass spectra obtained under different

conditions as described below.

### EXPERIMENTAL

A series of compounds has been made with different  
5 substituents at the phenyl rings of the core trityl structure. Some of these  
compounds are believed to be new and form additional aspects of this  
invention. The chemistry involved is illustrated in Figure 4 of the  
accompanying drawings. The compounds and their properties are as  
follows.

10

#### **Figure 4.**

a.  $\text{SOCl}_2$ , reflux. b. 2-amino-2-methylpropanol-1, 2.5 eqv.  
c. phenylmagnesium bromide. d. 80% AcOH, 48h. e. NHS, DCC.  
f. AcCl, toluene, reflux. g. Grignard synthesis. **3** is alternatively  
15 synthesised from benzophenone and 4-bromophenyl oxazoline.

### Example 1

***N-succinimidyl-4-[bis-(4-methoxyphenyl)-chloromethyl]-benzoate (1)***  
was synthesised according to the reported procedures (1,2).

20

***N-succinimidyl-4-[(4-methoxydiphenyl)-chloromethyl]-benzoate (2)***  
was synthesised according to the reported procedures (1,2), but  
4-methoxybenzophenone was used in the Grignard synthesis instead of  
4, 4'-dimethoxybenzophenone.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ,  $\delta$ , md): 7.95-6.8 (m, 13H,  
25 arom.), 3.8 (s, 3H,  $\text{OCH}_3$ ), 2.88 (s, 4H, NHS). Mass-spectrum, TOF (no  
matrix): ( $\text{M} + \text{H}^+$ ).

***N-succinimidyl-4-[bis-(phenyl)-chloromethyl]-benzoate (3)*** was  
synthesised according to the reported procedures (1,2), but benzophenone  
30 was used in the Grignard synthesis instead of  
4, 4'-dimethoxybenzophenone.  $^1\text{H-NMR}$  ( $\text{CDCl}_3$ ,  $\delta$ , md): 8.0-6.8 (m, 14H,

arom.), 2.88 (s, 4H, NHS). Mass-spectrum, TOF (no matrix): (MI + H<sup>+</sup>).

The formation of the Grignard reagent from 2-(4-bromophenyl)-4,4-dimethyl-1,3-oxazoline is rather unreliable and irreproducible, even using RED-Al®(Aldrich) as an activator. To take advantage of commercially available Grignard reagents (Aldrich), the inventors synthesised oxazolyl-protected 4-carboxybenzophenone (**4**) starting from 4-carboxy benzophenone. Following the Grignard reaction, subsequent steps were similar to those used for compounds **1-3**.

- 10 **2-(4-benzophenyl)-4,4-dimethyl-1,3-oxazoline (4)**. 4-benzoylbenzoic acid (50g, mmol) was refluxed in 300 ml of thionyl chloride for 3h, evaporated (the product crystallises from the oil) and then evaporated with toluene (2 x 30 ml). The residue was dissolved in 254 ml of dry methylene chloride. To this ice-cooled solution, 46g (2.5 eqv) of 2-amino-2-methylpropanol-1 in 150 ml of dry methylene chloride was added dropwise for 2h. The solution was stirred overnight at room temperature, and the precipitate was washed several times with methylene chloride. Combined fractions were evaporated, carefully dissolved in 350 ml of thionyl chloride and refluxed for 4h. The reaction mixture was evaporated to 1/3, poured into 2L of dry ether and kept overnight at 4°C. The precipitate of hydrochloride was dissolved in 1L of water at 10°C, and 300ml of 5M KOH was added to it with stirring. The mixture was extracted with chloroform (3 x 350ml), organic phase dried over CaCl<sub>2</sub> and evaporated. The crystalline product was obtained from toluene. 42g (75%) white crystalline solid, mp 81°C. <sup>1</sup>H-NMR (CDCl<sub>3</sub>, δ, md): 8.2-7.2 (m, 9H, arom.), 4.18 (s, 2H, CH<sub>2</sub>), 1.45 (s, 6H, CH<sub>3</sub>). Mass-spectrum, MALDI-TOF: 279.091 (MI), 302.085 (MI + Na<sup>+</sup>), 319.656 (MI + K<sup>+</sup>).

The masses of the majority of commercially available amines lay in the range of 50-250 Da. For some applications it would be desirable to have up to a few hundred mass-tags. The resolution of the tags in TOF mass-spectrometry was found to be satisfactory with at least 4 Da

difference between the masses of tags. Therefore, the above range of amines can only yield about 50 different tags. To increase this amount using the same pool of amines, the inventors synthesised another trityl-based compound with two activated carboxyl groups (6), which upon reaction with amine would form two amide bonds thus giving other series of mass-tags as compared, for example, to (2). Additional increase of mass can be achieved by attaching even more amines to trityl core by using, for example, (8).

10 **4,4'-[bis-(2-(4,4-dimethyl-1,3-oxazolyI))]-4''-methoxytritanol (5).** To 1.5g of magnesium turnings activated with iodine 15.34g (mmol) of bromophenyl oxazoline in 150ml of dry THF and a droplet of RED-A1® were added with stirring and the mixture was refluxed for 3h, cooled to room temperature and 4.64g (mmol) of methyl 4-methoxybenzoate in 40ml of dry THF was  
15 added dropwise. The mixture was gently refluxed for 6h, cooled to room temperature and 10 ml of water was added with stirring. Organic phase was carefully decanted and residue washed several times with small portions of THF. Combined organic fractions were evaporated and purified (flash-chromatography) to give 11.4g (84%) of light yellow solid. Mass-spectrum, MALDI-TOF: 467.545 (MI - OH), 484.869 (MI). <sup>1</sup>H-NMR (CDCl<sub>3</sub>,  
20 δ, md): 7.95-6.75 (m, 12H, arom.), 4.12 (s, 4H, CH<sub>2</sub>), 3.78 (s, 3H, OCH<sub>3</sub>), 1.37 (s, 12H, CH<sub>3</sub>).

**4,4'-[bis-(2-(succinimidylcarboxy))]-4''-methoxytrityl chloride (6).** The  
25 solution of 5 (10g, mmol) in 250ml of 80% acetic acid was kept at 72°C for 48h, evaporated and then evaporated with water (2 x 50 ml). The product was dissolved in 75ml of 50% EtOH/water, refluxed for 3h and evaporated to 1/3. The mixture was then dissolved in 100ml of water and acidified with 3M HCl to pH 1-2. The precipitate was dissolved in chloroform, dried  
30 (Na<sub>2</sub>SO<sub>4</sub>) and evaporated to dryness and additionally dried *in vacuo* overnight. Dicarboxylic acid obtained was dissolved in 100ml of dry THF.



8.5g (mmol) of N-hydroxysuccinimide was added and the mixture was cooled to 0°C. Dicyclohexylcarbodiimide (8.5g, mmol) in 20ml of dry THF was added dropwise with stirring. The reaction mixture was stirred 1h at 0°C and overnight at room temperature. Dicyclohexylurea was filtered off and organic phase was evaporated to dryness and purified (flash-chromatography) to give 8.5g (%) of white yellow-white solid. Mass-spectrum, MALDI-TOF: 554.703 (MI + OH), 570.794 (MI). <sup>1</sup>H-NMR (CDCl<sub>3</sub> δ, md): 8.2-6.75 (m, 12H, arom.), 3.81 (s, 3H, OCH<sub>3</sub>), 2.9 (s, 8H, CH<sub>2</sub>). This compound was converted into corresponding trityl chloride by refluxing in AcCl/toluene for 3h. The reaction mixture was then evaporated to 1/3. 2/3 of volume of toluene was added, the mixture was again evaporated to 1/3 and used without further purification.

To introduce a tagging moiety during oligonucleotide synthesis (Figure 4), we synthesised non-nucleoside phosphoramidite synthon **7** based on propanediol structure, which provides reactivity similar to the standard A, C, G and T phosphoramidites. The MMTr(NHS)Cl has reduced reactivity compared to both DMTrCl and DMTr(NHS)Cl, and it is important, when synthesising **7**, to carry out the tritylation reaction at low temperatures to prevent the formation of ester bond between excess of propanediol and the activated carboxyl group (data not shown). The phosphoramidite **7** was stable in acetonitrile solution at room temperature for at least 2 days.

***O<sup>1</sup>-[[4-(succinimidylcarboxy)]-4'-methoxytrityl]-1,3-propanediol.***

The title compound was synthesised according to a published procedure (4), but the tritylation step was carried out in pyridine at 0°C overnight without a catalyst. Monoprotected propanediol was obtained with a yield of 55% after flash-chromatography as a white foam.

Mass-spectrum, MALDI-TOF (α-cyano-4-hydroxycinnamic acid): 488.76 (MI). <sup>1</sup>H-NMR (CDCl<sub>3</sub>, δ, md): 8.15-6.8 (m, 13H, arom.), 3.8 (s, 3H, OCH<sub>3</sub>), 3.79 (t, 2H, CH<sub>2</sub>OH), 3.28 (t, 2H, J = 6 Hz, MMTr(NHS)OCH<sub>2</sub>), 2.9

(s, 4H, succinimide), 1.89 (quin, 2H, CH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>).

*O*<sup>1</sup>{[4-(succinimidylcarboxy)]-4'-methoxytrityl}-*O*<sup>3</sup>-  
(*N,N*-diisopropylamino-2-cyanoethoxy  
5 *phosphinyl*)-1,3-propanediol (7).

Phosphitylation of monoprotected propanediol was carried out as described in (4) to give the title phosphoramidite as a white foam in 70% yield. <sup>31</sup>P-NMR (CDCl<sub>3</sub>, δ, md): 144.512. Mass-spectrum, MALDI-TOF (dihydroxybenzoic acid): 689.934 (M).

10

**Analysis of trityl-based tags.** 0.1 M solutions of compounds 1, 2, 3 and 6 were prepared in the mixture of THF/dioxane (1:1). 110 µl of these solutions were mixed with 40 µl (80 µl in case of 6) of 0.5-1 M solutions of different amines (Table 1). The mixtures were then analysed with and  
15 without matrix either directly or when mixed in different combinations.

To evaluate these modified trityl blocks as precursors for mass-tags, compounds 1 and 2 were used to synthesise 5'-protected thymidine. 0.1 M solutions of these nucleosides and also of compounds 3 and 6 in OH-form (tritanols) in THF were reacted with 0.5-1 M solutions of  
20 amines in THF or dioxane (5eqv. of amine for mono-NHS-based compounds and 10 eqv. for 6), by mixing 200 µl of each of tritylated compounds with the corresponding amount of an amine solution and allowing them to react for 5 min. The reaction mixtures were then analysed by mass spectrometry without matrix, to prevent formation of molecular  
25 ions, by applying 1 µl of these mixtures directly onto a sample target plate and allowing them to dry. Typical results are presented in Table 1. For all trityl derivatives, the compounds which gave the strongest signal were selected and analysed as a mixtures by mixing all and applying 1 µl of the mixture to the target plate. Excellent results were achieved, both with and  
30 without matrix.

All synthesised trityl blocks were tested for acid-lability by

treatment with 2-5% TsOH or HClO<sub>4</sub> of corresponding 5'-thymidylates and TLC-analysis of the products after quenching with sat. sodium bicarbonate. As expected, there was about one order of magnitude difference in stability between DMTr, MMTr and Tr. Corresponding NHS-derivatives were about  
5 twice as stable, i.e. the stability was: DMTr < DMTr(NHS) < MMTr < MMTr(NHS) < Tr < Tr(NHS).

To be used as a tag in oligonucleotide synthesis, the trityl group should give clean high intensity signal in (MA)LDI-TOF analysis. It should also survive several steps of acidic treatment used to remove  
10 5'-DMTr group in oligonucleotide synthesis, that is, be orthogonal to other protective groups involved. (The NHS-group is stable to the conditions of oligonucleotide synthesis, see Example 2). The MMTr(NHS) group is preferred as the one to meet both these demands. It remained attached to a primary hydroxyl group after at least 5-8 cycles of acidic deprotection in  
15 oligonucleotide synthesis using twofold diluted standard solution of trichloroacetic acid in dichloromethane and a reduced deprotection time. For analysis, it was easily released using 3-4% TFA in the same solution (~1-3 min).

## 20 REFERENCES

1. Gildea, B.D., Coull, J.M. and Koster, H. (1990) *Tet. Lett.*, **31**, 7095-7098.
2. Coull, J.M., Gildea, B. and Koster, H. Pat. USA 5,410,068 (1995).
- 25 3. Gait, M.J. (1984) *Oligonucleotide Synthesis. A Practical Approach*. IRL Press, Oxford, UK.
4. Seela, F. and Kaiser, K. (1987) *Nucl. Acids Res.* **15**, 3113-3129.

**Table 1**

#	MW, Da	Chemical name	Observed Mass of Tritylamide (no matrix)			
			Tr(NHS) (3)	MMTr(NHS) (2)	DMTr(NHS) (1)	MMT <sub>2</sub> (NHS) (6)
1	17.03	Ammonia	286.27	316.25	346.31	359.36
2.	31.06	Methylamine	300.33	330.32	360.43	387.45
3.	45.09	Ethylamine	314.38	344.35	374.45	415.54
4.	59.11	Propylamine	328.39	358.44	388.53	443.64
5.	73.14	Butylamine	342.47	372.46	402.51	471.71
6.	74.09	Glycinamide (xHCl)	-----	372.44	403.60	474.55
7.	85.15	Cyclopentylamine	354.42	384.47	414.53	495.71
8.	87.17	Amylamine	356.43	386.60	416.52	499.73
9.	87.17	2-Amino-3-methylbutane	356.38	386.42	416.50	499.65
10.	89.14	2-Amino-1-methoxypropane	358.35	388.34	418.14	503.57
11.	89.14	4-Amino-1-butanol	358.40	388.39	418.41	503.65
12.	89.14	2-Amino-2-methyl-1-propanol	358.39	388.43	418.51	503.66
13.	97.12	Furfurylamine	366.43	396.42	426.54	519.64
14.	99.18	Cyclohexylamine	368.47	398.52	428.58	523.78
15.	101.19	Hexylamine	370.56	400.55	430.69	527.76
16.	103.17	5-Amino-1-pentanol	372.52	402.60	432.63	531.78
17.	103.19	Thiomorpholine	372.47	402.46	432.55	531.72
18.	105.14	2-(2-Aminoethoxy)-ethanol	374.45	404.53	434.56	535.66
19 -	113.20	Cycloheptylamine	382.50	412.49	442.58	551.86
20.	114.19	1-(2-Aminoethyl)-pyrrolidine	383.43	413.45	443.59	553.68
21.	115.22	Heptylamine	384.49	414.48	444.55	555.81
22.	121.18	Phenethylamine	390.52	420.48	450.58	567.63
23.	122.17	2-(2-Aminoethyl)-pyridine	391.62	421.54	451.63	569.84
24.	125.18	1-(3-Aminopropyl)-imidazole	394.24	424.58	454.73	576.34
25.	127.23	Cyclooctylamine	396.62	426.61	456.77	580.00
26.	128.18	$\alpha$ -Amino- $\epsilon$ -caprolactam	397.56	427.54	457.65	581.91
27.	128.22	2-(2-Aminoethyl)-1-methylpyrrolidine	397.64	427.67	457.71	-----
28.	128.22	1-(2-Aminoethyl)-piperidine	-----	427.62	457.69	581.91
29.	129.25	Octylamine	398.64	428.57	458.70	583.92
30.	130.19	4-(2-Aminoethyl)-morpholine	399.56	429.49	459.60	585.94
31.	130.23	N,N-Diethyl-1,3-propanediamine	399.53	429.55	459.66	585.85
32.	135.17	3-Phenylpropylamine	404.54	434.51	464.66	595.72
33.	135.21	1-(4-Methylphenyl)-ethylamine	404.59	434.55	464.65	595.89
34.	137.18	4-Methoxybenzylamine	406.57	436.55	466.63	599.85
35.	137.18	2-Phenoxyethylamine	406.61	436.55	466.56	599.91
36.	139.17	4-Fluoro-( $\alpha$ -methyl-benzylamine	408.62	438.58	468.63	663.91
37.	142.20	1-(3-Aminopropyl)-2-pyrrolidinone	411.63	441.67	471.77	610.02
38.	143.14	3,5-Difluorobenzylamine	412.56	442.55	472.67	611.85

39.	143.27	Nonylamine	412.67	442.65	472.74	612.10
40.	144.22	4-(3-Aminopropyl)-morpholine	413.57	443.52	473.58	-----
41.	147.22	1,2,3,4-Tetrahydro-1-naphthylamine	416.55	446.49	476.59	619.82
42.	149.06	2,2,3,3,3-Pentafluoropropylamine	418.50	448.46	478.58	623.78
43.	149.24	1-Methyl-3-phenylpropylamine	418.65	448.61	478.58	623.95
44.	149.24	4-Phenylbutylamine	418.64	448.61	478.73	623.95
45.	151.21	Norephedrin	420.62	450.63	480.77	627.98
46.	151.21	2-(4-Methoxyphenyl)-ethylamine	420.63	450.66	480.71	627.95
47.	151.25	1-Adamantylamine	420.69	450.62	480.75	628.06
48.	155.29	4-tert-Butylcyclohexylamine	424.73	454.68	480.75	636.06
49.	155.29	Menthylamine	424.68	454.63	484.77	635.82
50.	156.27	1-(3-Aminopropyl)-2-pipecoline	-----	455.63	485.79	-----
51.	157.22	1-Naphtalenemethylamine	426.52	456.49	486.55	639.69
52.	157.30	Decylamine	426.68	456.64	486.70	640.02
53.	158.29	2-Amino-5-diethylaminopentane	427.75	457.67	487.81	642.12
54.	160.22	Tryptamine	428.67	459.64	489.72	645.97
55.	162.24	1-Phenylpiperazine	431.69	461.70	491.75	649.99
56.	167.21	2,6-Dimethoxybenzylamine	436.63	466.60	496.65	659.94
57.	171.33	Undecylamine	440.85	470.76	500.88	668.21
58.	175.15	4-(Trifluoromethyl)-benzylamine	444.65	475.66	504.52	676.03
59.	176.26	1-Benzyl-3-aminopyrrolidine	445.72	474.53?	505.74	677.93
60.	180.23	1-(4-Fluorophenyl)piperazine	449.60	477.64	509.71	685.94
61.	181.25	Dicyclohexylamine	-----	-----	-----	-----
62.	183.25	Aminodiphenylmethane	452.60	482.57	512.63	691.80
63.	185.36	Dodecylamine	454.80	484.79	514.86	696.16
64.	190.29	4-Amino-1-benzylpiperidine	459.73	489.69	519.83	706.00
65.	191.27	2-Benzylloxycyclopentylamine	460.80	490.68	520.54	708.02
66.	193.15	3-Fluoro-5-(trifluoromethyl)-benzylamine	462.75	492.60	522.64	711.88
67.	197.28	1,2-Diphenylethylamine	466.75	496.68	526.83	719.96
68.	199.38	Tridecylamine	468.90	498.85	528.93	724.30
69.	200.26	4-(2-Aminoethyl)benzenesulfonamide	469.59	499.59	529.68	725.81
70.	206.75	1-(2-Ethoxyphenyl)piperazine (XHCl)	477.71	502.68	535.99	-----
71.	207.28	Amino-2,2-dimethyl-4-phenyl-1,3-dioxane	476.53	506.49	536.44	752.22
72.	213.41	Tetradecylamine	482.81	512.79	542.81	752.30
73.	221.31	9-(Methylaminomethyl)-anthracene	490.77	520.83	-----	-----
74.	227.44	Pentadecylamine	496.88	526.90	557.03	780.21
75.	231.30	1-Pyrenemethylamine	509.83	540.93	571.08	808.36
76.	241.46	Hexadecylamine	512.74	542.71	572.78	812.17
77.	243.15	3,5-Bis(trifluoromethyl)-benzylamine	-----	-----	-----	860.46
78.	269.50	Octadecylamine	538.99	568.92	599.15	864.52
79.	297.57	Didecylamine	566.97	596.99	627.02	920.55
80.	339.61	Hexetidine	609.00	638.99	-----	-----

### Example 2

An oligonucleotide trimer, 5'-GAA-3', was synthesised directly on PEG-grafted Rapp beads on 1  $\mu$ mol scale of synthesis using standard 3'-phosphoramidites. The beads were then subject to 4 more steps of oligonucleotide synthesis, this time according to split-and-mix strategy as outlined in Figure 2, using **7** and 16 different amines, thus producing a library of 256 different 7-mers. After ammonia deprotection the beads were washed and hybridised to 0.05  $\mu$ mol of 5'-Cy5-TTC.CAG.T (10) and 5'-Cy5-TTC.TAT.T (11) as described below.

ca 3-6 mol% of **7** was mixed with standard A, C, G and T phosphoramidites prior to oligonucleotide synthesis. Assuming the stepwise yield of oligonucleotide synthesis to be about 99%, for an 8-mer library synthesised using **7** as a 5% additive to all bases, that would give ca 60% of all sites of the beads occupied by full length oligonucleotides. The concentration of the first tag (5% of all initial sites) would be about two-fold greater than that of the last tag (5% of the remaining 60% of the sites), which still makes it possible to detect all of them in the same mixture. Oligonucleotide synthesis was carried out on a 4-column ABI machine. After each oxidation step, the columns were removed and treated with different amines. No particular rationale was used when choosing masses of tags to be used for encoding of particular base/position. But in principle, certain mass-ranges could be used to code for either a base type (i.e. all As are coded by tags ranging from 400 to 500 Da, all Cs- by tags in the interval of 500-600 Da, etc) or, alternatively, the position of the base might be a determining factor (i.e., position #1, or a 3'-end, (A, C, G or T) is coded by mass-tags ranging from 400-420 Da, position #2- by 420-440 Da, etc).

Beads were selected by hybridisation with Cy5-labelled oligonucleotide. The size of Rapp-beads (~0.3 mm) allows for manual removal of positively identified beads, visible with the naked eye, from the pool. For smaller beads, automated methods such as Flow-cytometry

(FACS), might be used. Selected beads were detritylated and the mixtures of tags released analysed by mass spectrometry.

To eliminate any problem of gradual loss of encoding MMT<sub>r</sub>-based tags during the detritylation step in oligonucleotide synthesis, 5'-Fmoc-protective strategy has also been used, thus omitting the use of acidic conditions altogether. After each oxidation step, the columns were removed from the synthesizer, and the beads were treated with corresponding amines washed with acetonitrile and then treated with 0.1 M DBU in acetonitrile to remove Fmoc-protection. The tags encoding for 9-mer oligonucleotide synthesised using this strategy were detected using (MA)LDI-TOF analysis. For longer sequences, the 3'-methylphosphoramidites of 5'-Fmoc-protected nucleosides could be preferably used instead of cyanoethoxy phosphoramidites, to prevent the untimely loss of the CNET- group due to the treatment with amines and DBU.

**Oligonucleotide synthesis.** Oligonucleotide synthesis was carried out using commercially available standard A, C, G and T, PAC, fluorescein and Cy5 phosphoramidites according to the manufacturer's protocols.

20

**Synthesis of the combinatorial library.** Phosphoramidite **7** was added to standard A,C,G and T phosphoramidites up to 3-6 mol.% of total amount of phosphoramidite. 35-40 mg (ca. 2500 beads) of Rapp TentaGel Macrobeads were placed in each of four polypropylene DNA synthesis columns (1  $\mu$ mol scale, Glen Research, USA). The oligonucleotide synthesis was carried out on 1  $\mu$ mol scale using phosphoramidite mixtures: A + **7**, C + **7**, G + **7** and T + **7**, according to the manufacturer's protocol, but the supply of deblocking reagent (diluted to 50% of its original concentration with methylene chloride) to the columns was reduced to 10-15 sec, with subsequent waiting step (10 sec) and another portion of acid (10 sec). Subsequent thorough acetonitrile washing of the columns

30

ensured that all DMTR+ is desorbed. Before each detritylation step, the columns were washed with acetonitrile using Manual Control mode, and then treated with corresponding amines (0.3-0.5 ml of 0.5-1 M solutions in dioxan/THF, depending on the solubility of an amine) for 1 min using 1 ml syringes. The columns were then washed excessively with acetonitrile and dried *in vacuo* for 15 min. The beads from all columns were then combined together in a 0.3 ml reaction vial with conical chamber (Pierce), mixed and then split again in four portions by pipetting the suspension of beads in acetonitrile (about 1 volume of solvent per 1 volume of beads) using a 1 ml Eppendorf tip. The procedure was repeated till the end of the synthesis. The beads were then washed, dried, deprotected for 14 h in 1.5 ml of concentrated ammonia at 55°C, then washed several times with distilled water, dried and stored at 4°C.

**Hybridisation of the combinatorial library and detritylation.** The beads were hybridised to 5'-Cy5-labelled 7-mer oligonucleotides 5'-TTC.CAG.T (10) and 5'-TTC.TAT.T (11) in 1.5 ml of 3.5 M TMA buffer (51) in a 4 ml vial which was rotated on a Spiramix 10 machine overnight at 8°C. The beads were then washed 5 times with TMA buffer at the same temperature, transferred onto a surface of a 7.5 x 5 cm microscope slide and the excess of the buffer removed by blotting with tissue paper. Coloured or otherwise identified beads were then removed using tweezers (usually about 30-50 beads), washed with water, acetone and dried. The trityl tags were cleaved by treating the beads with 0.08-0.1 ml of 3-4% (v/v) solution of trifluoroacetic acid in standard Deblok Solution (Cruachem; trichloroacetic acid in dichloromethane) for 3-4 min. Supernatant was evaporated several times with acetone or methanol to remove the acids and the residue was analysed by (MA)LDI-TOF. Excellent quality results were obtained.



### **Example 3**

Thiolated oligonucleotides have previously been used to immobilise PCR products onto gold monolayers (e.g. Hegner *et al.*). The attachment is due to bonding between the gold and the thiol group. Using  
5 this chemical reaction it is possible to immobilise any gene, or region of any gene, onto the gold plated surface of a mass spectrometer target plate, via a thiol linkage.

This example illustrates the immobilisation of PCR products to the gold surface of mass spectrometer target plates; hybridisation and  
10 ligation of pairs of oligonucleotides, one of which defines the locus and the other a putative allele; the allele is characterised by the detection of a trityl tag(s) by mass spectrometer. The example given uses M13mp18 ssDNA as a putative target region.

#### **PCR and immobilisation (Figure 5)**

A 225 base pair PCR product was amplified from M13mp18 ssDNA using two oligonucleotide primers;  
A1 5'ACTGGCCGTCGTTTTAC3'- ; B1 5'AAGGGCGATCGGTGCGG 3'-.  
A1 was synthesised with the addition of a 17 atom linker molecule, and a  
20 thiol group, to the 5' end using conventional phosphoramidite chemistry (see figure ). The thiol group was activated with a 200 fold excess of DTT, and 5ng of product was spotted on to the gold target plate. Incubation in 100% humidity overnight was sufficient to immobilise the PCR product. Excess template was removed by flooding the plate with 10mM Tris-HCl  
25 (pH 7.5).

#### **Hybridisation and ligation (Figure 6)**

The oligonucleotide defining the locus,  
C1 - 5'GTAAAACGACGGCCAGT3' was synthesised with a phosphate  
30 group coupled to the 5' end. Two putative allele defining oligonucleotides were synthesised, D1 and D2- 5'CACGACGTT3' differing only in their 5'

terminus where D1 was tagged with dimethoxytrityl and D2 with monomethoxytrityl. Both oligonucleotides were synthesised using conventional phosphoramidite chemistry and were fully complementary to the PCR amplified product.

5                   Ligation and hybridisation was at 46°C (Housby and Southern, 1998) overnight in 100% humidity, and under saturating concentrations of oligonucleotides C1 and D1 and/or D2. For ligation, a thermostable DNA ligase, *Tth*, was used because of its high temperature optimum for ligation and high degree of discrimination for the 3' end of  
10                   substrate oligonucleotide. Residual unligated oligonucleotides were removed by washing.

#### **Mass spectrometer analysis**

                  The mass spectrum of monomethoxytrityl (MMT, mass 272)  
15                   clearly demonstrated cleavage of MMT from the ligated product. No matrix was used to assist ionisation. Control samples showed no detectable peaks at 272.

                  The same experiment using dimethoxytrityl as the tagged oligonucleotide demonstrates that DMT also "flies" well and has a peak at  
20                   303 mass units.

                  Derivatised T residues have been coupled with aminated DMT and MMT derivatives as described. A selection of DMT tagged T residues have been mixed together and analysed by mass spectrometry (see figure 7.)

25

#### **Example 4**

                  A disposable glass insert, for use as a target surface for laser desorption ionisation mass spectrometry was manufactured from 1 mm plain soda glass, cut to a rectangle 45mm x 46mm, and the cut edges  
30                   bevelled. A standard gold-plated metal insert (Perkin Elmer/Perseptive Biosystems part number VES 503 405) may be used as a template to

position orientation markings on the reverse of the glass insert prior to assembly. The insert was held in a disposable sample plate holder (Perkin Elmer/Perseptive Biosystems part number VES 700 314). The 4 retaining screws of the sample holder were removed, the glass insert placed into the upper rectangular frame from behind, and the sample holder was re-assembled. Samples for analysis may be applied using existing methods. Perkin Elmer/Perseptive Biosystems "Voyager"™ Biospectrometry™ Workstation software version 4.03 may be used to correctly locate samples on the plate, and analysis was performed using existing methods.

10                   The mass spectrum shown in Figure 8 was produced by application of the manufacturer's recommended mass calibration mixture to a glass target. The mixture (Perkin Elmer/Perseptive Biosystems part number 2-3243-00-000) comprises 3 peptides des-arg1-bradykinin, angiotensin 1 and glu1-fibrinopeptide B. Analysis was performed using a standard method. The theoretical mass of the singly protonated ion is 15                   904.4681 Da, 1296.6853 Da and 1570.6774 Da respectively

### **Example 5**

#### **Polypropylene**

20                   *In situ* synthesis of oligonucleotides directly on to the surface of polypropylene has been described in detail (Matson *et al*, 1994; Southern *et al*, 1994). Surface synthesis of a specific short (10-20 bases) sequence can be used to capture a target sequence. Once captured, allele specific oligonucleotides (ASO) can be hybridised to the captured target 25                   sequence. Subsequent ligation of a mass tagged oligonucleotide would be used in mass spectrometry to define the allele. Mass spectrometry is enabled by securing the polypropylene array into a disposable sample plate holder in a similar manner to that described in Example 4.

30                   A 9-mer oligonucleotide with DMT left 'on' at the end of conventional phosphoramidite DNA synthesis, was spotted (2000 pmol) on to a piece of polypropylene. Laser Desorption/Ionisation Time of Flight

Mass Spectrometry, without matrix revealed a peak at 309.3 mass units. The mass of DMT is 303 mass units. The higher observed mass is due to the polypropylene (strip) target being placed behind the disposable target plate, thereby increasing the ion flight length. The plate was used to keep  
5 the polypropylene strip secured for mass spectrometry.

### Example 6

#### **Epoxysilane Chemistry**

Glass targets are prepared for mass spectrometric analysis  
10 as described in Example 4. The surface is activated by treatment with epoxysilane (Beattie *et al*, 1995) and a target DNA is synthesised with a terminal amine at either the 3' or 5' end. 5  $\mu$ M of aminated oligonucleotide is spotted on to the surface of the activated glass plate and left from 2 hours to overnight. The surface is washed in H<sub>2</sub>O at 60°C, 10 mM  
15 triethylamine, pH 9.2, at room temperature, followed by 2 washed in hot water. Hybridisation of a probe oligonucleotide, labelled with a <sup>32</sup>P radioisotope, is in 3M TMAC, 60 mM Tris pH 7.5, 6 mM EDTA, 0.03% SDS, for 1 hour, followed by a 1 hour wash in the same buffer. Analysis of the hybridisation efficiency can be determined by phosphorimager analysis.  
20 Ligation and mass spectrometric analysis of tagged oligonucleotides will determine the allele.

#### **Isothiocyanate Chemistry**

This method has been described previously (Weiler and  
25 Hoheisel, 1997). For the purpose of these experiments, glass target plates are silanized by immersion in 10% NaOH overnight followed by washing in H<sub>2</sub>O and methanol. The plates are then sonicated in 3% aminopropyltrimethoxysilane in methanol, followed by washing in water followed by methanol. The plates are then dried in nitrogen and baked at  
30 110°C for 15 minutes.

For the immobilisation of aminated oligonucleotides the plates

are activated by phenylendiisothiocyanate (PDITC) (Weiler and Hoheisel, 1997). Target oligonucleotides are synthesised with a 5' or 3' amine. Immobilisation of the target DNA is carried out in 0.001 M NaOH, 0.1 – 1.0  $\mu$ M oligonucleotide, for 2 hours, at room temperature. Hybridisation of <sup>32</sup>P radiolabelled complementary probe oligonucleotides is carried out in 3 x SSPE and 0.5% SDS for 1 hour followed by washing in the same buffer and subsequent phosphorimager analysis. Ligation and mass spectrometric analysis of tagged oligonucleotides will determine the allele.

#### 10 **Mercaptosilane Modified Glass Surface**

This method for the derivitisation of a mercaptosilane modified glass surface has been described previously (Rogers *et al*, 1999). Briefly, glass target plates are cleaned overnight in 25% ammonium hydroxide, rinsed in water for 10 minutes followed by a brief rinse in anhydrous ethanol. For silanisation, the glass plates are immersed in a 1% solution of 3-mercaptopropyl trimethoxysilane (MPTS), 95% ethanol, 16 mM acetic acid (pH 4.5), for 30 minutes. Finally, the plates are rinsed in 95% ethanol / 16 mM acetic acid (pH 4.5) and dried in a vacuum oven for 2 hours at 150°C.

20 Target DNA is immobilised via a thiol / disulphide exchange reaction. The thiol group can be readily added to the 5' end of any oligonucleotide using standard phosphoramidite chemistry. Typically, 10  $\mu$ M of thiolated oligonucleotide, in 500 mM NaHCO<sub>3</sub> (pH 9.0), is spotted on to the activated mercaptosilane surface, for 2 hours to overnight. The surface is then washed in TNTw buffer (10 mM Tris-HCl, pH 7.5, 150 mM NaCl and 0.05% Tween 20).

A 40 base 5' thiolated oligonucleotide has been immobilised to a mercaptosilanised glass target. Hybridisation of a <sup>32</sup>P radiolabelled 17 base complementary probe sequence, has demonstrated high specificity for the complementary target sequence with little background hybridisation.

Exempl 7**Polylysine**

A 27-mer oligonucleotide was attached to a glass mass spectrometry target pre-treated with polylysine. After treatment to inhibit  
5 non-specific binding, specific hybridisation of a complementary 14-mer with high efficiency has been successfully demonstrated.

A glass MS target (described in Example 4) was cleaned and treated with polylysine solution (Sigma P8920) following the general procedure described below:

- 10 1. Place slides in slide racks. Place racks in chambers.
2. Prepare CLEANING SOLUTION: Dissolve 70 g NaOH in 280 ml ddH<sub>2</sub>O. Add 420 ml 95% ethanol. Total volume is 700 ml (=2 x 350 ml); stir until completely mixed. If solution remains cloudy, add ddH<sub>2</sub>O until clear.
- 15 3. Pour solution into chambers with slides; cover chambers with glass lids. Mix on orbital shaker for 2 hrs. *Once slides are clean, they should be exposed to air as little as possible. Dust particles will interfere with coating and printing.*
4. Quickly transfer racks to fresh chambers filled with ddH<sub>2</sub>O .
- 20 Rinse vigorously by plunging racks up and down. Repeat rinses 4x with ddH<sub>2</sub>O. *It is critical to remove all traces of NaOH-ethanol.*
5. Prepare POLYLYSINE SOLUTION: 70 ml poly-L-lysine + 70 ml tissue culture PBS in 560 ml water. *Use plastic graduated cylinder and beaker.*
- 25 6. Transfer slides to polylysine solution and shake for 15 min - 1 hr.
7. Transfer rack to fresh chambers filled with ddH<sub>2</sub>O . Plunge up and down 5x to rinse.

- 32 -

3 oligonucleotides were prepared using standard methods

1a            5' Cy-GCAGTCAGTC ACAGAAGGTG TTTCTGA 3'  
1b            5'        GCAGTCAGTC ACAGAAGGTG TTTCTGA 3'  
5    2            5' Cy-GAAACACCTT CTGT 3'

Oligo 1a is labelled with fluorescent cyanine dye, oligo 1b is the same sequence unlabelled. Oligo 2 is also cyanine labelled and is complementary in sequence to bases 11 to 24 of oligos 1a and 1b.

10            2-fold serial dilutions of oligos 1a and 1b were prepared in deionised distilled water from 500 ng/ $\mu$ l to 1 ng/ $\mu$ l. Two duplicate 1  $\mu$ l spots of each dilution were placed on the glass target and allowed to dry in air. The glass target was treated to inhibit non-specific DNA binding following the general procedure described below:

- 15    1.            Place arrays in slide rack. Have empty slide chamber ready on orbital shaker.
2.            Prepare BLOCKING SOLUTION: Dissolve 5.5 g succinic anhydride in 325 ml 1-methyl-2-pyrrolidinone. Immediately after succinic anhydride dissolves, add 25 ml sodium borate.
- 20    3.            Immediately after sodium borate solution mixes in, pour solution into empty slide chamber. Plunge slide rack in solution several times. Mix on orbital shaker 15-20 min. Meanwhile, heat ~700 ml water (enough to cover slide rack) to 95°C in 2l beaker.
4.            Gently plunge slide rack in 95°C water for 2 min.
- 25    5.            Plunge slide rack 5x in 95% ethanol.

Fluorescence was scanned and recorded using a STORM860 fluorescence reader to confirm attachment of oligo 1a. Attachment of the unlabelled oligo 1b of the same sequence is inferred.

30            Hybridisation solution (4 x SSC, 0.25% SDS, 240 pmol oligo 2) was prepared in a total volume of 52  $\mu$ l, boiled for 2 minutes, placed on

- 33 -

to the glass target and covered with an untreated glass target. Hybridisation proceeded for 8 hours in a humid chamber containing 3 x SSC at room temperature. The target was then rinsed 3 times in 2 x SSC, briefly rinsed in deionised distilled water and allowed to dry in air.

- 5 Attachment of the labelled oligo 2 to the immobilised, unlabelled complementary sequence oligo 1b was confirmed by fluorescence scanning.

### Data

- 10 Following treatment to inhibit non specific binding, fluorescence of bound oligo 1a was detected from 500 ng/μl. Post hybridisation, fluorescence of bound oligo 2 was detected across the complete range of dilutions from 500 ng/μl to 1 ng/μl. Non-specific background fluorescence is acceptable.

15

### Example 8

#### Solution Experiment

- A solution experiment was performed to test the ligation of tagged oligonucleotides to template DNA. 9 base oligonucleotides were either tagged or not. A matched oligonucleotide had a mass tag of 413.5 units, and a mismatched oligonucleotide had a mass tag of 402 units. A 17 base primer was used to direct the ligation process. The reaction comprised of combinations of each 9-mer oligonucleotide. The concentrations of template and tagged oligonucleotides was 120 fmol whilst the 17 base primer was at a concentration of 60 fmol. The primer was radiolabelled with <sup>32</sup>P using polynucleotide kinase. Each reaction contained 50 units of *Thermus thermophilus* DNA ligase and was incubated for four hours at either 37°C or 46°C. The reaction was stopped with formamide and the products analysed by polyacrylamide gel electrophoresis.
- 20
- 25
- 30

The resulting ligated band was excised from the gel and the



DNA eluted by incubation in a high salt buffer at 37°C overnight. The DNA was then purified and analysed by mass spectroscopy without matrix. The resulting mass of 413.5 corresponded to the mass tag used for the ligation of the fully complementary oligonucleotide, N. There was no detectable  
5 peak at 402 mass units corresponding to the mismatched sequence of oligonucleotide M. This indicates that the ligation reaction is highly specific for the correctly matched oligonucleotide and is therefore confirmation that the procedures will be adaptable for high throughput genotyping.

10

### Example 9

#### **Abstract**

Glass slides were silanised with (3-mercaptopropyl) trimethoxysilane in dry toluene. The terminal thiol function was then reacted with 2,2'-dipyridyl disulfide to form a pyridyl disulfide linkage. A  
15 43-mer oligodeoxynucleotide, modified at its 5'-end with a terminal phosphorothioate group was attached to the modified slides.

Untreated glass slides were soaked in a solution of 5% (v/v) (3--mercaptopropyl)trimethoxysilane in dry toluene for 6 hours. The slides were washed with dry toluene followed by ethanol. The slides were  
20 subsequently soaked overnight in a 6.66 g/l solution of 2,2'-dipyridyl disulfide in isopropanol. The slides were finally washed isopropanol and air dried.

The oligonucleotide had the sequence:

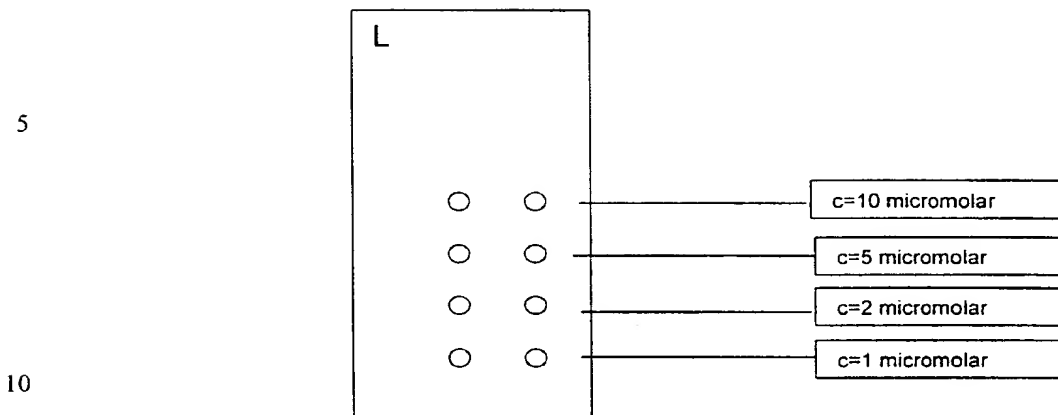
5' -p(s) -TTT TAG CAA TGG GCA GTC AGT CAC AGA AGG TGT TTC  
25 TGA GAC C 3'

with p(s) = terminal thiophosphate

Oligonucleotide solutions of 40 µM, 20 µM, 8 µM and 4 µM were prepared in water.

To 20 µl of each dilution, 20 µl 0.8 M Citrate buffer pH 4, 20 µl  
30 ethyleneglycol and 40 µl water were added.

Final concentrations were 10 µM, 5 µM, 2 µM and 1 µM.



1  $\mu$ l oligonucleotide solution was applied on each spot. The slides were kept at room temperature for 18 hours, washed with water followed by isopropanol and were allowed to dry.

15

### Example 10

***O*<sup>1</sup>-{[4-(succinimidylcarboxy)]-4'-methoxytrityl}-6-trifluoroacetamido-1-hexanol (2).** A solution of *N*-succinimidyl-4-*bis*-(phenyl)-chloromethylbenzoate (1) (0.8g, 1.7 mmol) in pyridine (25ml) at ambient temperature was treated with an excess of 6-trifluoroacetamido-1-hexanol (0.91g, 4.25mmol), which was added in small portions as a solid. The resulting solution was stirred overnight at ambient temperature under an atmosphere of nitrogen. The solvent was then removed under vacuum to give a pale yellow oil. Flash column chromatography (5:1 dichloromethane – ethyl acetate) afforded the title compound as a white foam. Yield = 0.39g (35%).

$\delta$  (300MHz, CDCl<sub>3</sub>) 8.02(2H, d, Aryl), 7.61(2H, d, Aryl), 7.39 – 7.20(9H, m, Aryl), 6.82(2H, d, Aryl), 6.27(1H, br, amide), 3.78(3H, s, OCH<sub>3</sub>), 3.32(2H, m), 3.03(2H, m), 2.87(4H, s, succinimidyl), 1.62 – 1.49(6H, m), 1.42 – 1.21(6H, m); IR (film)  $\nu$  3345, 1770, 1736, 1708 cm<sup>-1</sup>; MS (ES+) 644(M+H<sub>2</sub>O)<sup>+</sup>.

20

25

30

**O<sup>1</sup>-{[4-(succinimidylcarboxy)]-4',4''-dimethoxytrityl}-6-**

**trifluoroacetamido-1-hexanol (3)** was prepared from *N*-succinimidyl-4-[(4-methoxydiphenyl)-chloromethyl]-benzoate (**4**) (1.2g, 2.5mmol) and 6-trifluoroacetamido-1-hexanol (1.33g, 6.25mmol) using the procedure described above. Yield = 1.02g (62%) as a white foam.  $\delta_H$  (300MHz, CDCl<sub>3</sub>) 8.02(2H, d, Aryl), 7.61(2H, d, Aryl), 7.24(4H, d, Aryl), 6.81(4H, d, Aryl), 6.26(1H, br, amide), 3.78(6H, s, OCH<sub>3</sub> x 2), 3.32(2H, m), 3.02(2H, m), 2.86(4H, s, succinimidyl), 1.6 – 1.5(7H, m), 1.36 – 1.21(5H, m); IR (film)  $\nu$  3400, 1770, 1740, 1708 cm<sup>-1</sup>; MS (ES+) 697(M+MeCN)<sup>+</sup>.

**O<sup>1</sup>-{[4-(butylamidocarboxy)]-4'-methoxytrityl}-6-trifluoroacetamido-1-hexanol (5).**

A solution of O<sup>1</sup>-{[4-(succinimidylcarboxy)]-4'-methoxytrityl}-6-trifluoroacetamido-1-hexanol (0.39g, 0.63mmol) in acetonitrile (5ml) was treated with an excess of neat *n*-butylamine (0.18g, 2.5mmol, 0.25ml). The solution was stirred overnight at ambient temperature under an atmosphere of nitrogen. The solvent was then removed under vacuum and the residue partitioned between ethyl acetate and water. The organic layer was separated, dried over Na<sub>2</sub>SO<sub>4</sub> and filtered. Concentration of the filtrate gave a clear oil which crystallised on standing. Yield = 0.35g (96%).

$\delta_H$  (300MHz, CDCl<sub>3</sub>) 7.64(2H, d, Aryl), 7.49(2H, d, Aryl), 7.38(2H, d, Aryl), 7.27 – 7.20(5H, m, Aryl), 6.4(1H, br, amide), 6.13(1H, br, amide), 3.77(3H, s, OCH<sub>3</sub>), 3.41(2H, m), 3.29(2H, m), 3.02(2H, m), 1.57 – 1.50(7H, m), 1.38 – 1.21(5H, m), 0.91(3H, t, CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>N); IR (film)  $\nu$  3303, 3083, 1707, 1637, 1543, 1508 cm<sup>-1</sup>.

**O<sup>1</sup>-{[4-(butylamidocarboxy)]-4',4''-dimethoxytrityl}-6-**

**trifluoroacetamido-1-hexanol (6)** was prepared from O<sup>1</sup>-{[4-(succinimidylcarboxy)]-4', 4''-dimethoxytrityl}-6-trifluoroacetamido-1-hexanol (0.5g, 0.76mmol) and butylamine (0.22g, 3.0mmol, 0.3ml) using the procedure described above. Yield = 0.45g (95%) as a clear oil.

$\delta_H$  (300MHz, CDCl<sub>3</sub>) 7.64(2H, m, Aryl), 7.49(1H, d, Aryl), 7.35(1H, d, Aryl),

7.34 – 7.27(3H, m, Aryl), 7.13(2H, d, Aryl), 6.79(4H, m, Aryl), 6.36(1H, br, amide), 6.05(1H, br, amide), 3.77(6H, s, OCH<sub>3</sub> x 2), 3.63(1H, m), 3.46 – 3.28(6H, m), 1.55(7H, m), 1.37(4H, m), 0.91(4H, m); IR (film)  $\nu$  3301, 3033, 1708, 1637, 1544, 1508 cm<sup>-1</sup>.

5

**O<sup>1</sup>-[[4-(butylamidocarboxy)]-4'-methoxytrityl]-6-amino-1-hexanol (7).**

A solution of O<sup>1</sup>-[[4-(butylamidocarboxy)]-4'-methoxytrityl]-6-trifluoroacetamido-1-hexanol (0.35g, 0.6mmol) in methanol (5ml) was treated with 0.88spg aqueous ammonia (1ml) at ambient temperature. The solution was stirred overnight and the solvent then removed under vacuum. Analysis by thin layer chromatography (4:1 dichloromethane – ethyl acetate) showed the presence of a new, polar material which gave positive results when tested for the presence of an amino group (ninhydrin) and trityl residues (anisaldehyde / acid). The crude material was used without further purification.

15

**O<sup>1</sup>-[[4-(butylamidocarboxy)]-4',4''-dimethoxytrityl]-6-amino-1-hexanol (8)** was prepared from O<sup>1</sup>-[[4-(butylamidocarboxy)]-4',4''-dimethoxytrityl]-6-trifluoroacetamido-1-hexanol (0.45g, 0.7mmol) and aqueous ammonia (1ml) using the procedure described above.

20

**O<sup>1</sup>-[[4-(butylamidocarboxy)]-4'-methoxytrityl]-6-succinamido-1-hexanol (9).** O<sup>1</sup>-[[4-(butylamidocarboxy)]-4'-methoxytrityl]-6-amino-1-hexanol (~0.3mmol) was dissolved in anhydrous pyridine (5ml) at ambient temperature. Succinic anhydride (0.07g, 0.7mmol) was then added and the resulting solution stirred at ambient temperature for 2 hours. The solvent was then removed under vacuum and the residue analysed by t.l.c (4:1 dichloromethane – ethyl acetate). A new material was observed (R<sub>f</sub> 0.3) which gave a positive result when tested for trityl residues (anisaldehyde / acid), but did not give a positive result when tested for the presence of an amine (ninhydrin). The crude product was used without further purification.

25  
30

***O*<sup>1</sup>-{[4-(butylamidocarboxy)]-4', 4''-dimethoxytrityl}-6-succinamido-1-hexanol (10)** was prepared from *O*<sup>1</sup>-{[4-(butylamidocarboxy)]-4', 4''-dimethoxytrityl}-6-amino-1-hexanol (~0.35mmol) and succinic anhydride (0.09g, 0.9mmol) using the procedure described above. The product was used without further purification.

***N*-succinimidyl {*O*<sup>1</sup>-[4-(butylamidocarboxy)]-4'-methoxytrityl}-6-succinamido-1-hexanoate (11).** A solution of *O*<sup>1</sup>-{[4-(butylamidocarboxy)]-4'-methoxytrityl}-6-succinamido-1-hexanol and *O*-(*N*-succinimidyl)-*N,N,N',N'*-tetramethyluronium tetrafluoroborate in acetonitrile was treated with *N,N*-(diisopropyl)ethylamine while stirring at ambient temperature. After stirring for 2 hours the solvent was removed under vacuum and the residue subjected to flash column chromatography (98:2 dichloromethane – methanol). A partially purified product was obtained which was then further refined using preparative thin layer chromatography, eluting with 9:1 dichloromethane – methanol. The plate was allowed to dry thoroughly and then eluted again. A band corresponding to a trityl active material on t.l.c was removed, triturated with 9:1 dichloromethane – methanol. The silica was filtered off and the filtrate concentrated under vacuum to give a small amount of the title compound. Yield = 10mg.

$\delta_H$  (300MHz, *d*<sub>3</sub>-MeCN) 7.68(2H, d, Aryl), 7.49(2H, d, Aryl), 7.42(2H, d, Aryl), 7.33 – 7.23(5H, m, Aryl), 7.1(1H, br, amide), 6.86(2H, d, Aryl), 6.6(1H, br, amide), 3.75(3H, s, OCH<sub>3</sub>), 3.34 – 3.27(2H, m), 3.10(2H, m), 2.99(2H, m), 2.56(4H, s), 2.55 – 2.38(4H, m), 1.56 – 1.49(3H, m), 1.40 – 1.29(7H, m), 1.21(1H, m), 0.91(3H, t, CH<sub>3</sub>(CH<sub>2</sub>)<sub>3</sub>N).

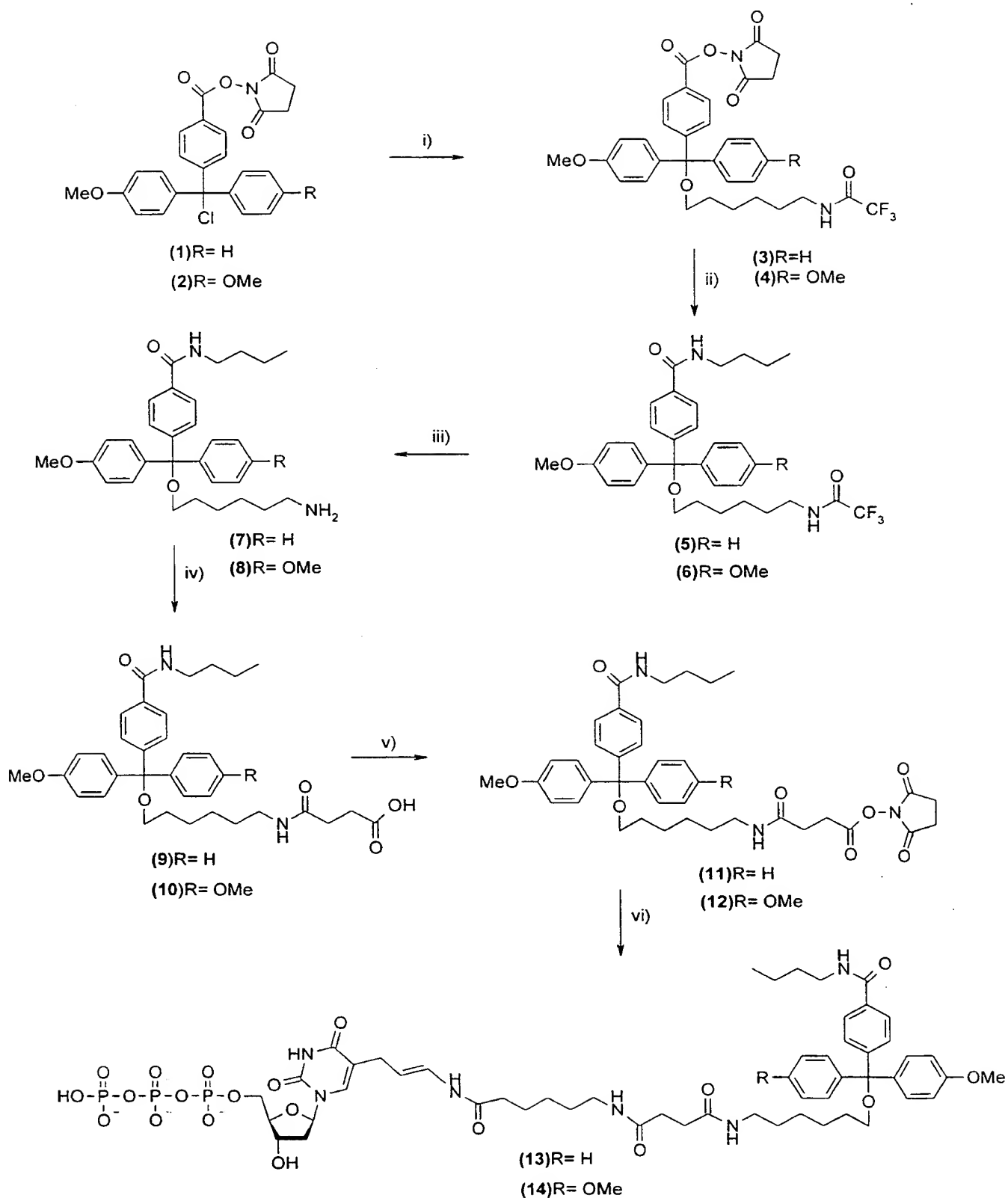
***N*-succinimidyl {*O*<sup>1</sup>-[4-(butylamidocarboxy)]-4', 4''-dimethoxytrityl}-6-succinamido-1-hexanoate (12)** was prepared from *O*<sup>1</sup>-{[4-(butylamidocarboxy)]-4', 4''-dimethoxytrityl}-6-succinamido-1-hexanol (~0.3mmol), *O*-(*N*-succinimidyl)-*N,N,N',N'*-tetramethyluronium

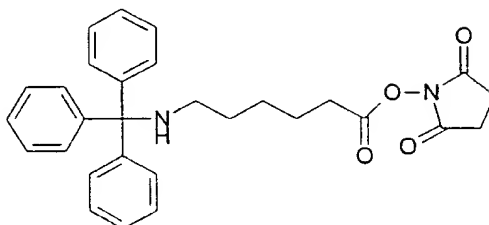
- 39 -

tetrafluoroborate (0.27g, 0.91mmol) and *N,N*-(diisopropyl)ethylamine (0.24g, 1.9mmol, 0.33ml). The product was obtained impure.

Yield = 0.045g.

Both of the above compounds were tested for the presence of the active ester by preparing dilute solutions in dichloromethane and then adding an excess of *n*-butylamine. The reactions were examined by t.l.c (9:1 dichloromethane – methanol) and in both cases showed the complete conversion of the starting material to a more polar product. Reaction of the activated ester with amino nucleotide derivatives could also be expected to result in the formation of similar amide-linked products.



**Example 11****6-*N*-Tritylamino succinimidylhexanoate**

5

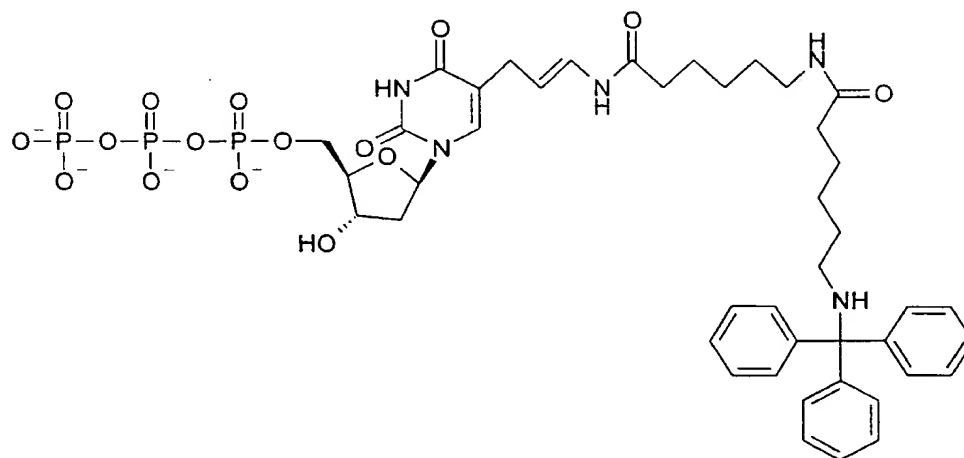
*N*-Tritylamino caproic acid (214 mg, 0.57 mmol), dicyclohexylcarbodiimide (154 mg, 0.75 mmol), 4-*N*-dimethylaminopyridine (21 mg, 0.17 mmol), and *N*-hydroxysuccinimide (86 mg, 0.75 mmol) were dissolved in dry dioxane (5 ml). The reaction mixture was stirred overnight at room temperature. Dicyclohexylurea was filtered off, and the filtrate was  
10 evaporated to dryness and purified by flash chromatography (gradient of dichloromethane to 2% methanol / dichloromethane). Pale yellow foam isolated (270mg, 98%).

<sup>1</sup>H NMR (CDCl<sub>3</sub>, δ, md): 7.46-7.08 (m, 15H, arom), 2.74  
15 (s, 4H, succinimide), 2.53 (t, 2H, NH-CH<sub>2</sub>), 2.06 (t, 2H, COCH<sub>2</sub>), 1.86-1.16 (m, 6H, 3 x CH<sub>2</sub>).

Mass spectrum (Electrospray, + ve mode) 471.53 [M+H]<sup>+</sup>, 243.28 [Trityl]<sup>+</sup>.



**5-(Allyl-3-biscapromidyl-15-*N*-tritylamino)-2'-deoxyuridine-5'-triphosphate**



5

5-(Allyl-3-capromidyl-9-amino)-2'-deoxyuridine-5'-triphosphate (2.5 mg in 500  $\mu$ l carbonate buffer pH 8.5) was syringed into an Eppendorf with a microstirrer bar. Acetonitrile was added (125  $\mu$ l),

6-*N*-tritylamino succinimidylhexanoate (6mg in 45 $\mu$ l acetonitrile) was then added, followed by addition of more acetonitrile (45  $\mu$ l). The solution was stirred at room temperature in which the reaction mixture initially went turbid, then clear on further stirring. After 5 hours stirring the reaction mixture was purified on a silica prep. plate with concentrating zone (5:4:1 propan-2-ol: ammonia: water). Silica containing product was scraped from the plate and extracted with the same eluent system, evaporated, redissolved in 50:50 acetonitrile:water and filtered through a cotton wool plug. The solution was evaporated to a fine white powder. TLC after purification indicated the product was higher running than the starting triphosphate and also stained brown on strong heating with anisaldehyde (indicative of sugars).

20

Mass spectrum (Electrospray, + ve mode) 1014.11[M+Na]<sup>+</sup>, 243.28 [Trityl]<sup>+</sup>. (-ve mode) 990.35 [M-H]<sup>-</sup>, 910.18 [M-H<sub>2</sub>PO<sub>3</sub>]<sup>-</sup>.

Conjugates of allylamino-dUtp such as biotin, haptens, dyes and metal chelates have been reported in the literature as substrates for incorporation by polymerases.

5

### **Example 12**

#### **Overview**

A system of ligating mass-tagged oligo nucleotides to anchored complementary DNA sequences on a solid support via a hybridising third oligo is described. The system uses *Tth* DNA ligase as an enzymatic component, which catalyses the ligation of a DMT-modified oligo (the "reporter" sequence) onto a hybridising "probe" oligo. The probe oligo, applied in solution, is localised by hybridisation to tethered "template" oligos which remain attached to the solid support throughout all manipulations and detection. Hybridisation of neither the probe nor the reporter oligos will occur at elevated temperatures due to their short length, but the ligated product, with consequent higher  $T_m$ , is retained as wash temperatures are raised. The success of the ligation is dependent on the correct mismatch detection of the 3' base on the reporter against the corresponding base on the anchored template. *Tth* ligase will not catalyse the reaction in the presence of a mismatched base at this position.

15  
20

#### **Attachment chemistry**

Oligos have been specifically attached to derivatised glass surfaces using a variety of available and developed chemistries. Oligos with a terminal 5' amine group have been used in conjunction with isothiocyanate and epoxide derivatised glass, while thiol-modified oligos have been anchored using mercaptopropyl silane and pyridyl disulphide methods. This latter method, is the subject of the experiments described in this report.

25  
30

### Hybridisation experiments

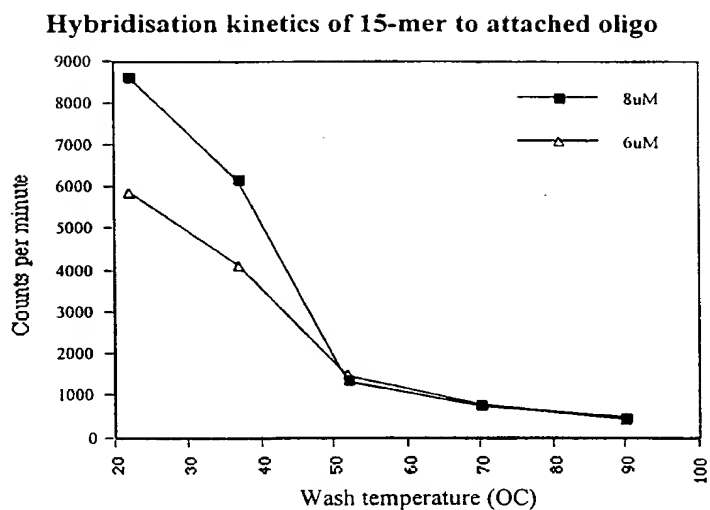
The following oligo sequences were used:-

5' TTTTAGCAATGGGCAGTCAGTCACAGAAGGTGTTTCTGAGACC 3' (template)  
5 TCAGTCAGTGTCTTCCACAAAGAC\* (reporter with mass tag)  
(probe)

The probe and reporter oligos have  $T_m$  values of 42 and 26°C respectively, allowing both to anneal at the experimental temperature of 22°C. Successive washing at higher temperatures reduces the amount of annealed primer, demonstrated in Chart 1 for the 15-mer probe, with the greatest reduction occurring in the region of the oligo's  $T_m$ . This pattern is consistent for both concentrations of oligo analysed (8 and 6µM). This indicates that hybridisation is the primary mode of interaction between the probe and template with good signal-to-noise ratios occurring for the higher concentrations of template.

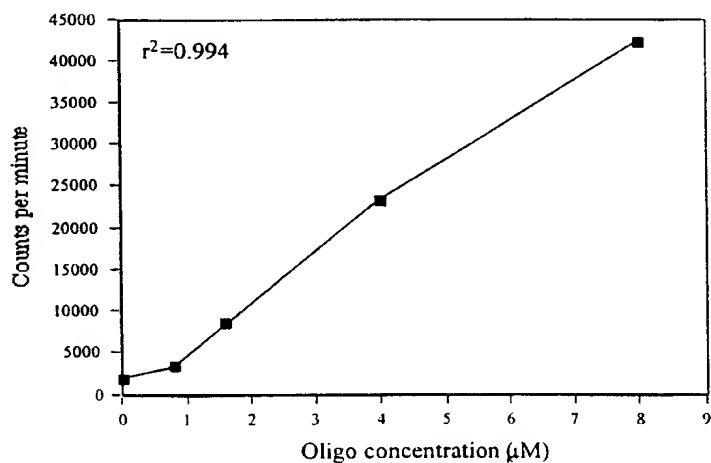
Chart 2 demonstrates a decrease in signal intensity as template deposition concentrations are lowered. There is considerable variability in the pyridyl disulphide attachment chemistry such that at lower template concentrations hybridization intensity is not consistent.

Demonstration of ligation of both probe and reporter oligos onto the same template and detection by mass spectrometry are continuing.



**Chart 1.** Effect of increased stringency washes on annealing of the 15mer probe oligo to attached template (conditions 1xTth ligation buffer; 0.1%SDS)

**Hybridisation of radiolabeled 15-mer to 5'-attached oligo**



**Chart 2.** Increase in hybridisation signal of labelled probe is proportional to attached template concentration. (sample washes in 1xTth ligase buffer; 0.1%SDS at 22°C)

## REFERENCES

- Beattie, W G; Meng, L; Turner, S L; Varmer, R S; Dao, D D  
and Beattie, K L (1995) Hybridization of DNA Targets to Glass-Tethered  
5 Oligonucleotide Probes. *Molecular Biotechnology*, **4**, 213-225.
- Chen, D; Yan, Z; Cole, D L and Srivatsa, S G (1999)  
Analysis of internal (n-1) mer deletion sequences in synthetic  
oligodeoxyribonucleotides by hybridization to an immobilized probe array.  
*Nucleic Acids Res.*, **27**, (2), 3889-995.
- 10 Hegner, M., Wagner, P., and Semenza, G. (1993) *FEBS* 336:  
452-456.
- Housby, J.N, and Southern, E.M. (1998) *Nucl. Acids. Res.*  
Submitted.
- Matson, R S; Rampal, J B and Coassin, P J (1994)  
15 Biopolymer synthesis on polypropylene supports. 1. Oligonucleotides.  
*Anal. Biochem*, **220**, (1), 225.
- Rogers, Y -H; Jiang-Baucom, P; Huang, J.-Z; Bogdanov, V;  
Anderson, S and Boyce-Jacino, M T (1999) Immobilization of  
Oligonucleotides onto a Glass Support via Disulphide Bonds: A Method for  
20 Preparation of Microarrays. *Anal Biochem*. **266**, 23-30.
- Southern, E M; Case-Green, S C; Elder, J K; Johnson, M;  
Mir, K U; Wang, L and Williams, J C (1994) Arrays of complementary  
oligonucleotides for analysing the hybridisation behaviour of nucleic acids.  
*Nucleic Acids Res.*, **22**, (8), 1368-1373.
- 25 Weiler, J and Hoheisel, J D (1997) Picomole syntheses of  
high quality oligonucleotide primers in combination with the preparation of  
oligonucleotide arrays. *Nucleosides and Nucleotides*, **16**, (7-9), 1793-  
1796.

### CLAIMS

- 5 1. A method of making a set of labelled compounds, by the use of a support and a set of labels, which method comprises the steps:
- a) at least one first or intermediate step comprising dividing the support into lots, performing a different chemical reaction on each lot of the support so as either to modify that lot of the support or to couple a chemical moiety to that lot of the support, tagging a fraction of each lot of the support with a different label, and combining the said lots of the support, and
- 10 b) at least one intermediate or final step comprising dividing the support into lots, performing a different chemical reaction on each lot of the support, so as either to modify that lot of the support or to couple a chemical moiety to that lot of the support, tagging a fraction of each lot of the support with a different label, whereby each different label is linked to a chemical moiety coupled to the support in a different step and forms with that chemical moiety a labelled compound which is separable from the support, and combining the said lots of the support.
- 15 2. The method of claim 1, wherein the support is a particulate solid support.
3. The method of claim 1 or claim 2, wherein step b) is
- 20 performed to couple the chemical moiety to a chemical moiety previously coupled to the support.
4. The method of claim 3, wherein the chemical moieties are monomer units and the labelled compounds are oligomers.

5. The method of claim 4, wherein the set of labelled compounds is a library of  $n^s$  oligomers, where  $n$  is the number of different monomer units and  $s$  is the number of monomer units in each labelled oligomer, wherein step a) is performed once to couple a different monomer unit to each lot of the support, and step b) is performed  $s-1$  times.
6. The method of claim 5, wherein the set of labelled compounds contains  $n \times s$  different labels.
- 10 7. The method of any one of claims 1 to 6, wherein each labelled compound comprises a single label and at least one chemical moiety.
8. The method of any one of claims 1 to 7, wherein the support  
15 is treated to release the said labelled compounds into solution.
9. The method of any one of claims 1 to 8, wherein from 0.25% to 25% of each lot of the support is tagged in each step with a different label.
- 20 10. The method of any one of claims 1 to 9, wherein the support has cleavable linkers, wherein each cleavable linker has at least one group for chemical synthesis and another group for labelling.
- 25 11. The method of any one of claims 1 to 10, wherein the label is joined by a link that is cleavable to give a charged species for mass spectrometry.

12. The method of any one of claims 1 to 11, wherein each label is a group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.

5

13. The method of claim 12, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary  
10 amido, anhydride, carbonyl halide or active ester.

14. The method of any one of claims 1 to 13, wherein the labelled compounds are labelled oligonucleotides.

15 15. A set of labelled compounds wherein a molecule of a compound of the set is tagged with a single label which identifies the nature and/or the position of a component of that molecule, and different molecules of the same compound are tagged with different labels.

20 16. The set of claim 15, wherein the labelled compounds are releasably attached to a solid support.

17. The set of claim 16, wherein the solid support is particulate.

25 18. The set of claim 15, wherein the labelled compounds are mixed together in solution.

19. The set of any one of claims 15 to 18, wherein the label is joined by a link that is cleavable to give a charged species for mass  
30 spectrometry.



20. The set of any one of claims 15 to 19, wherein each label is a group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.

5

21. The set of claim 20, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary  
10 amido, anhydride, carbonyl halide or active ester.

22. The set of any one of claims 15 to 21, wherein the labelled compounds are labelled oligonucleotides.

15 23. A library consisting of a plurality of the sets of any one of claims 19 to 22.

24. A reagent comprising a solid support which carries on its surface molecules of an oligomer, with different oligomer molecules having  
20 the same sequence wherein the oligomer molecules include some shorter oligomer molecules and a shorter oligomer molecule carries a label which identifies the nature and position of a monomer unit of the oligomer molecule.

25 25. The reagent as claimed in claim 24, wherein the solid support is a bead.

26. The reagent as claimed in claim 24 or claim 25, wherein the label is joined by a link that is photocleavable to give a charged species for  
30 mass spectrometry.

27. The reagent of any one of claims 24 to 26, wherein each label is a group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.

5

28. The reagent of any one of claims 24 to 27, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester.

10

29. The reagent of any one of claims 24 to 28, wherein the oligomers are oligonucleotides.

15

30. A library consisting of a plurality of the reagents of any one of claims 24 to 29.

31. A method, which method comprises providing a labelled oligonucleotide or nucleic acid, and removing the label by cleavage to give a charged species which is subjected to mass spectrometry.

20

32. The method of claim 31, wherein nucleic acid sequencing is performed by the use of a labelled primer and/or a labelled hybridisation probe and/or labelled chain extending nucleotides and/or labelled chain terminating nucleotide analogues, wherein the label is one which is removed by cleavage to give a charged species which is subjected to mass spectrometry.

25

33. An assay method in which a labelled probe is partitioned into two fractions one of which is determined, the probe comprising a ligand joined to a label by a link which is cleavable to give a charged species for mass spectrometry.

5

34. The method of claim 33, wherein the ligand is an oligonucleotide.

35. The method of any one of claims 31 to 34, wherein the label  
10 is a group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.

36. The method of claim 35, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$   
15 carries a substituent selected from  $C_1-C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester.

20 37. A library of probes each comprising a ligand joined to a label by a link which is cleavable to give a charged species for analysis by mass spectrometry, wherein each different probe has a different label.

38. The library of claim 37, wherein the ligand is an  
25 oligonucleotide.

39. The library of claim 37 or claim 38, wherein each label is a  
group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different  
and each is a monocyclic or fused ring aromatic group that is substituted or  
30 unsubstituted.

40. The library of claim 39, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary  
5 amido, anhydride, carbonyl halide or active ester.

41. A compound of formula  $R^1R^2R^3CY$ , where Y is a leaving group for reaction with a nucleophilic species, and  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group, at  
10 least one of which carries a substituent selected from  $C_1 - C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester, provided that  $R^1$ ,  $R^2$  and  $R^3$  together carry at least two amide groups and/or  
15 at least two N-hydroxysuccinimide ester groups.

42. The method of any one of claims 12, 13, 35 and 36, wherein  $R^1R^2R^3C-$  is a substituted monomethoxytrityl group.

20 43. The set of claims 20 or 21, or the reagent of claim 27 or 28, or the library of claims 41 or 42, wherein  $R^1R^2R^3C-$  is a substituted monomethoxytrityl group.

44. The compound of claim 41, wherein  $R^1R^2R^3CY$  is a  
25 substituted monomethoxytrityl compound.

45. An insert for use as a target for laser desorption ionisation mass spectrometry, which insert has a target surface of glass or of an organic polymer carrying an immobilised compound for analysis.

46. The insert of claim 45, wherein the target compound comprises a group of formula  $R^1R^2R^3C-$ , where  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group that is substituted or unsubstituted.
- 5
47. The insert of claim 46, wherein at least one of  $R^1$ ,  $R^2$  and  $R^3$  carries a substituent selected from  $C_1$ - $C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary
- 10 amido, anhydride, carbonyl halide or active ester.
48. The insert of claim 46 or 47, wherein  $R^1R^2R^3C-$  is a substituted monomethoxytrityl group.
- 15 49. The insert of any one of claims 45 to 48, wherein the target surface carries an array of immobilised compounds for analysis.
50. The insert of anyone of claims 45 to 49, wherein compounds are immobilised on target surfaces of glass by means of epoxysilane
- 20 chemistry or isothiocyanate chemistry or mercaptosilane chemistry or polylysine.
51. A kit comprising a mass spectrometer and a supply of inserts, for use as targets for laser desorption mass spectrometry, having target
- 25 surfaces of glass or of an organic polymer.

52. A system for analysing nucleic acids comprising:
- a solid support carrying an array of nucleic acids to act as targets for analysis or as probes to capture a target;
  - oligonucleotide reagents tagged with moieties suitable for analysis by mass spectrometry;
  - reagents and apparatus for biochemical procedures to allow specific interaction between the tagged oligonucleotides and the target;
  - a means to introduce the samples into a mass spectrometer;
  - a mass spectrometer.
53. A system for analysing nucleic acids on a solid support comprising:
- a solid support carrying an array of nucleic acids to act as targets for analysis or as probes to capture a target;
  - oligonucleotide reagents, tagged with moieties suitable for analysis by mass spectrometry;
  - reagents and apparatus for biochemical procedures to allow specific interaction between the tagged oligonucleotides and the target carried out on the solid support surface;
  - a means to introduce the solid support into a mass spectrometer;
  - a mass spectrometer.
54. An automated system for analysing nucleic acids comprising:
- oligonucleotide reagents, tagged with moieties suitable for analysis by mass spectrometry;
  - a mass spectrometer;
  - a computer to carry out the analysis;
  - software to interpret a mass spectrum.

55. A nucleotide or oligonucleotide labelled with a tag suitable for analysis by mass spectrometry, said labelled nucleotide or oligonucleotide being suitable for enzymatic incorporation, wherein the tag is a compound of formula  $R^1R^2R^3CY$ , where Y is a leaving group for reaction with a nucleophilic species, and  $R^1$ ,  $R^2$  and  $R^3$  are the same or different and each is a monocyclic or fused ring aromatic group, at least one of which carries a substituent selected from  $C_1 - C_{20}$  alkoxy or hydrocarbyl either unsubstituted or substituted by carboxylic acid, sulphonic acid, nitro, cyano, hydroxyl, thiol, primary, secondary or tertiary amino, primary or secondary amido, anhydride, carbonyl halide or active ester.

56. The method of any one of claims 31 to 36, wherein 4s different labels are used, where the labelled oligonucleotide or nucleic acid contains s bases and each label is indicative of the position and identity of a nucleotide residue of the labelled oligonucleotide or nucleic acid.

57. The method of claim 56, wherein for a base position of the labelled oligonucleotide or nucleic acid, four regions of a mass spectrum corresponding to the masses of the four possible labels (including their isotopic variants) are examined and compared with an expected mass spectrum of the label.

58. The method of any one of claims 31 to 36, wherein each possible oligonucleotide or nucleic acid containing s bases is compared in turn against a mass spectrum comprising the s different tag regions, to identify the oligonucleotide having the best fit.

1/8

Fig.1A.

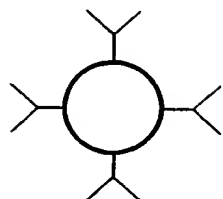


Fig.1B.

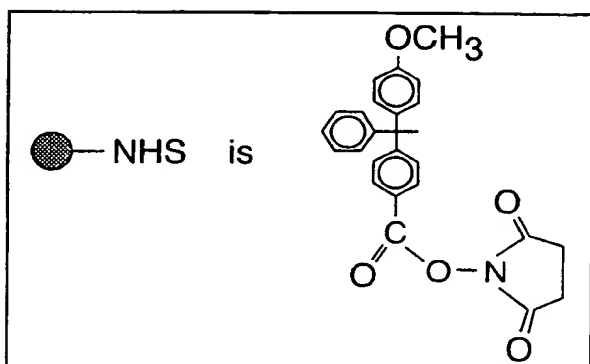
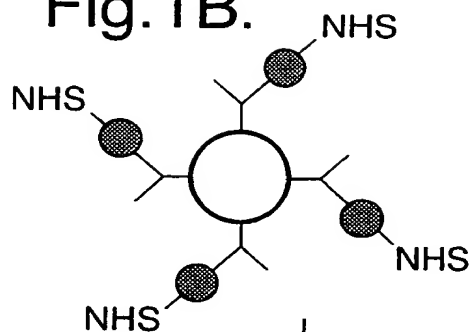


Fig.1C.

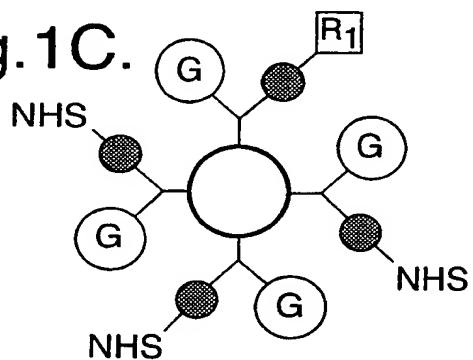


Fig.1D.

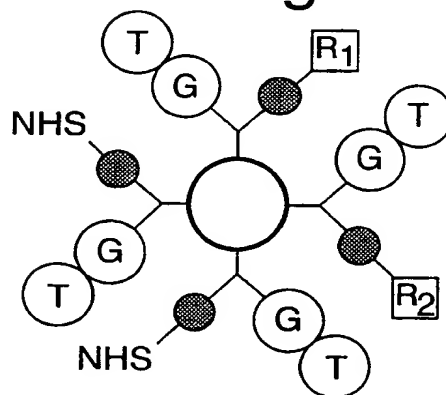


Fig.1E.

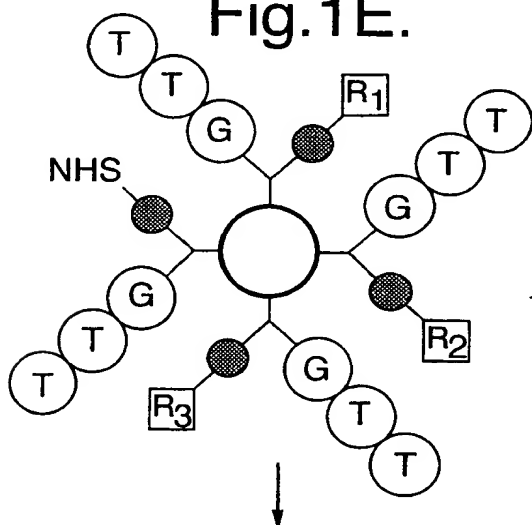


Fig.1F.

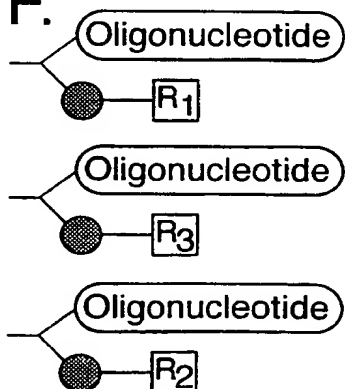


Fig.1G.

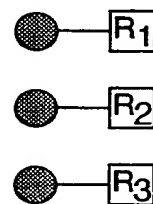




Fig.2A.

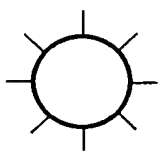


Fig.2B.

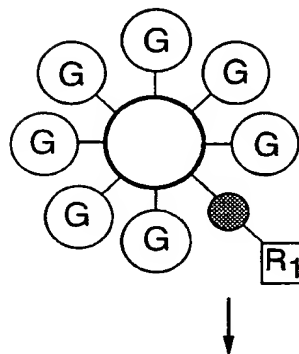


Fig.2D.

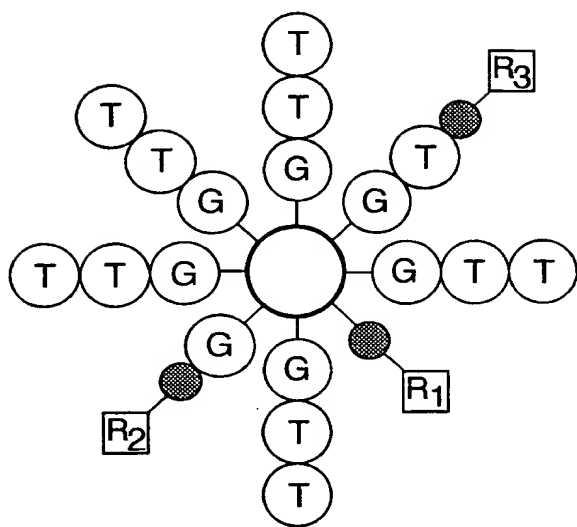


Fig.2C.

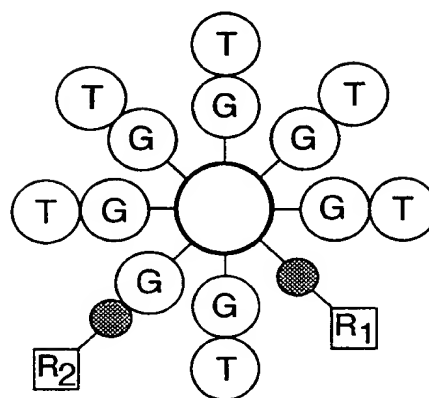


Fig.2E.

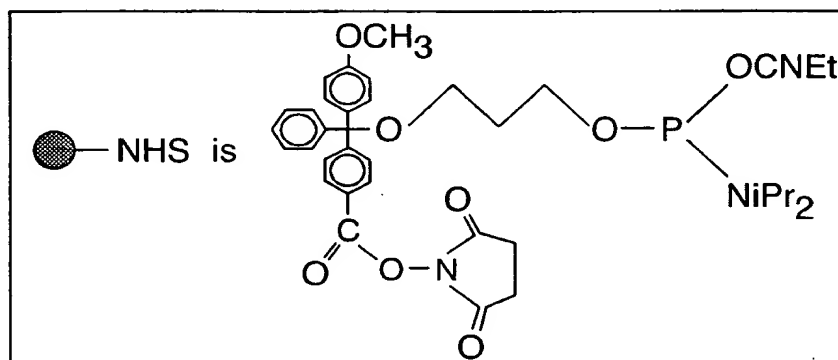
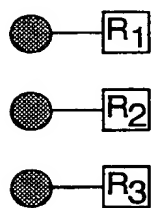
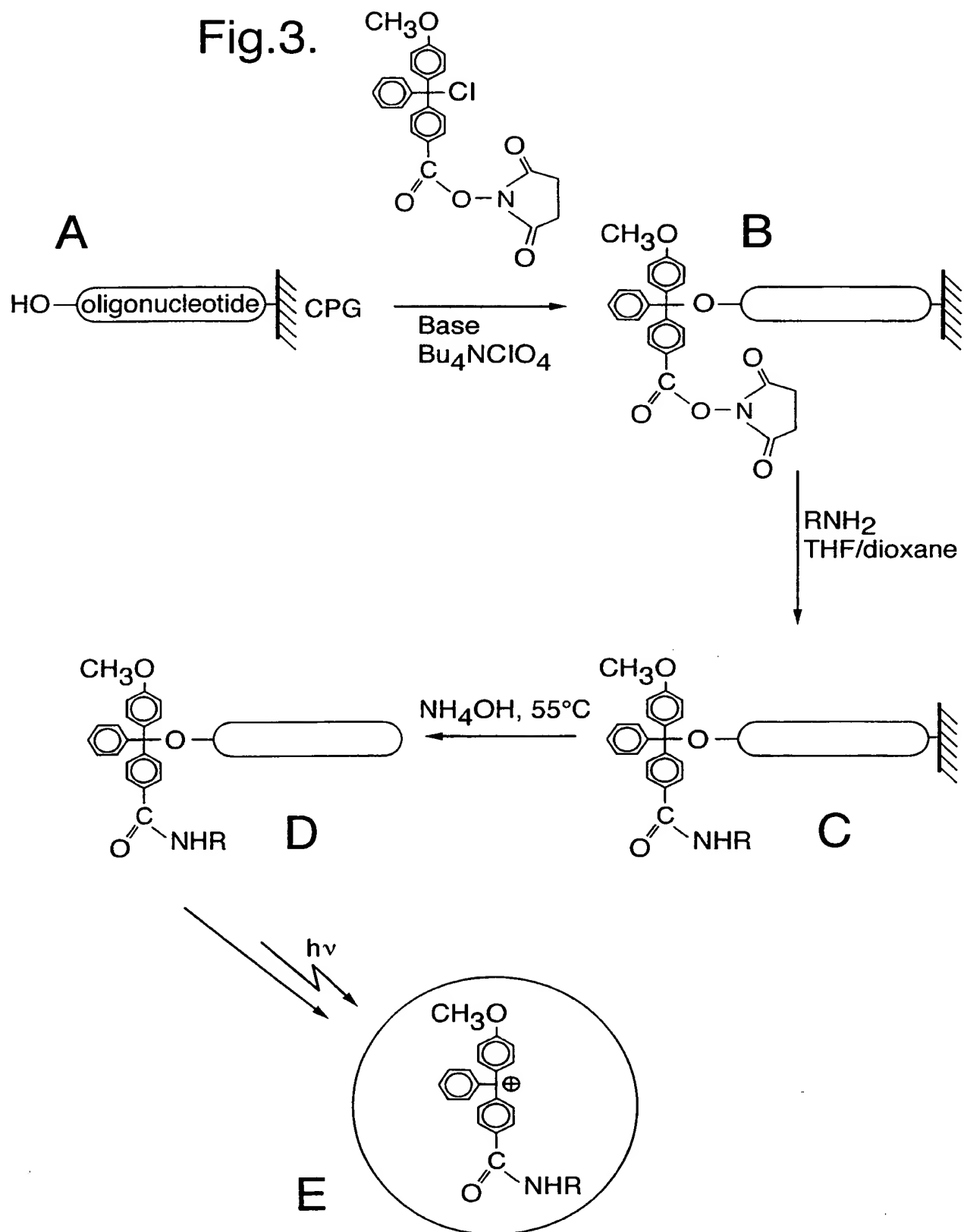
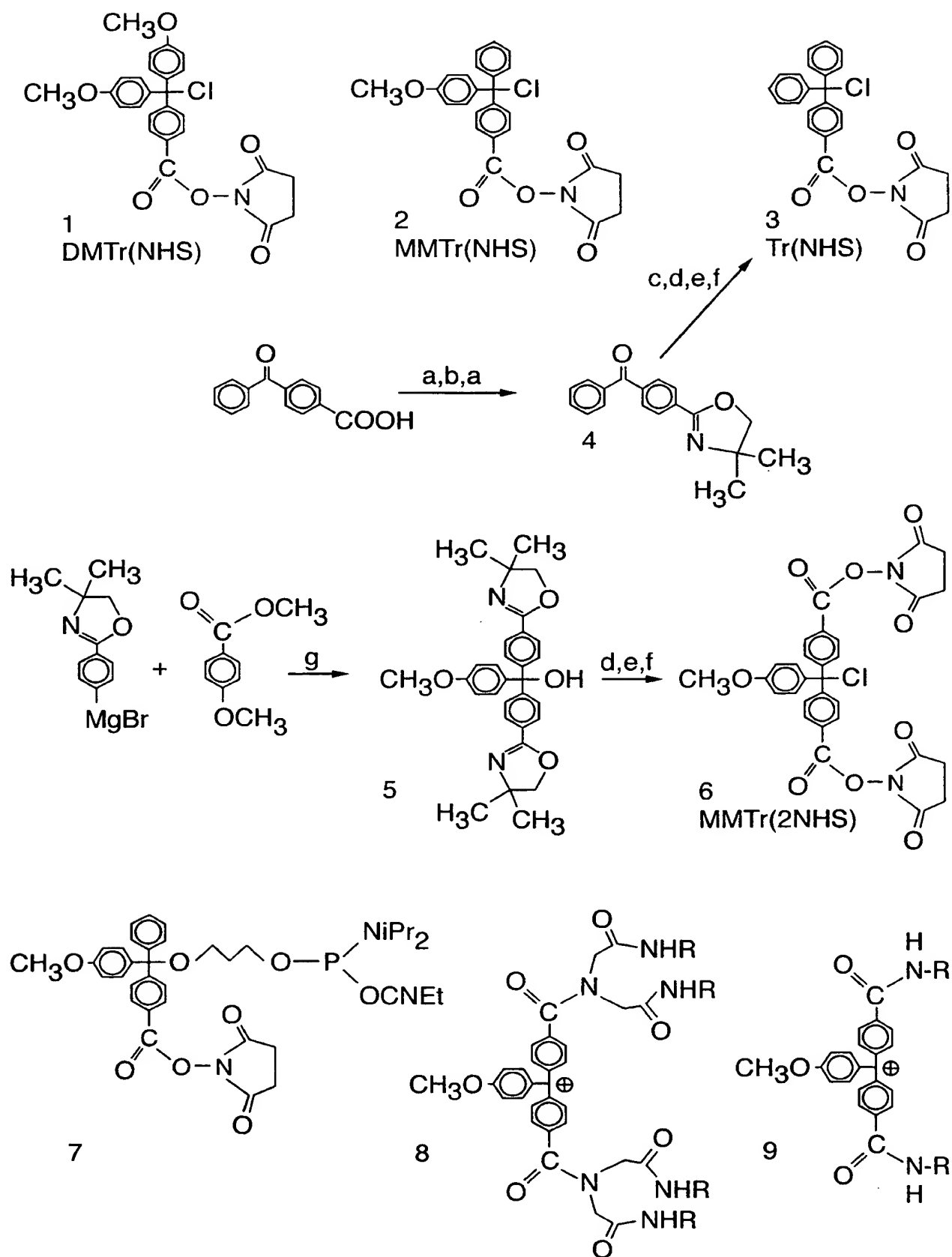


Fig.3.



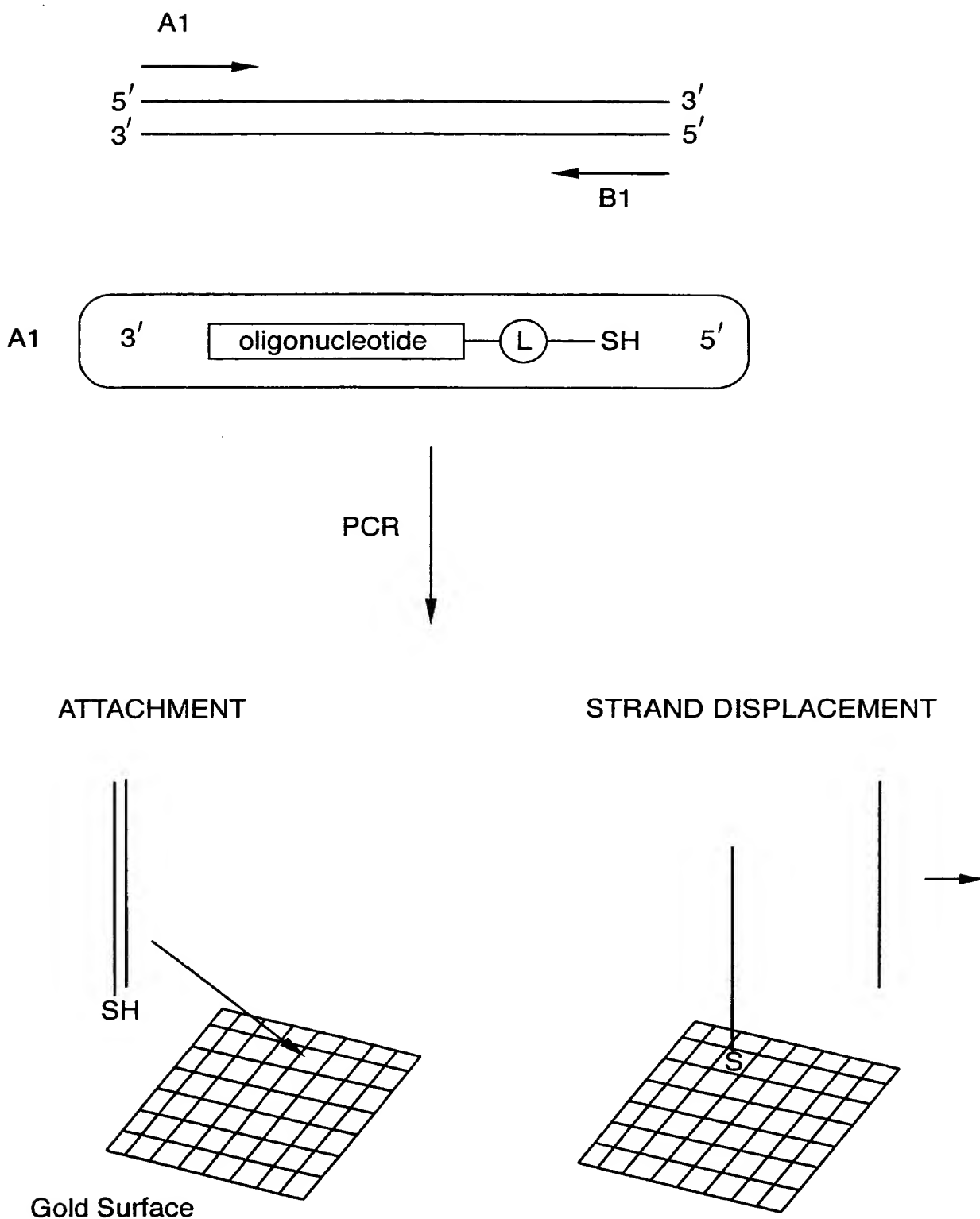
4/8

Fig.4.



5/8

Fig.5.



# Fig.6.

## HYBRIDISATION AND LIGATION

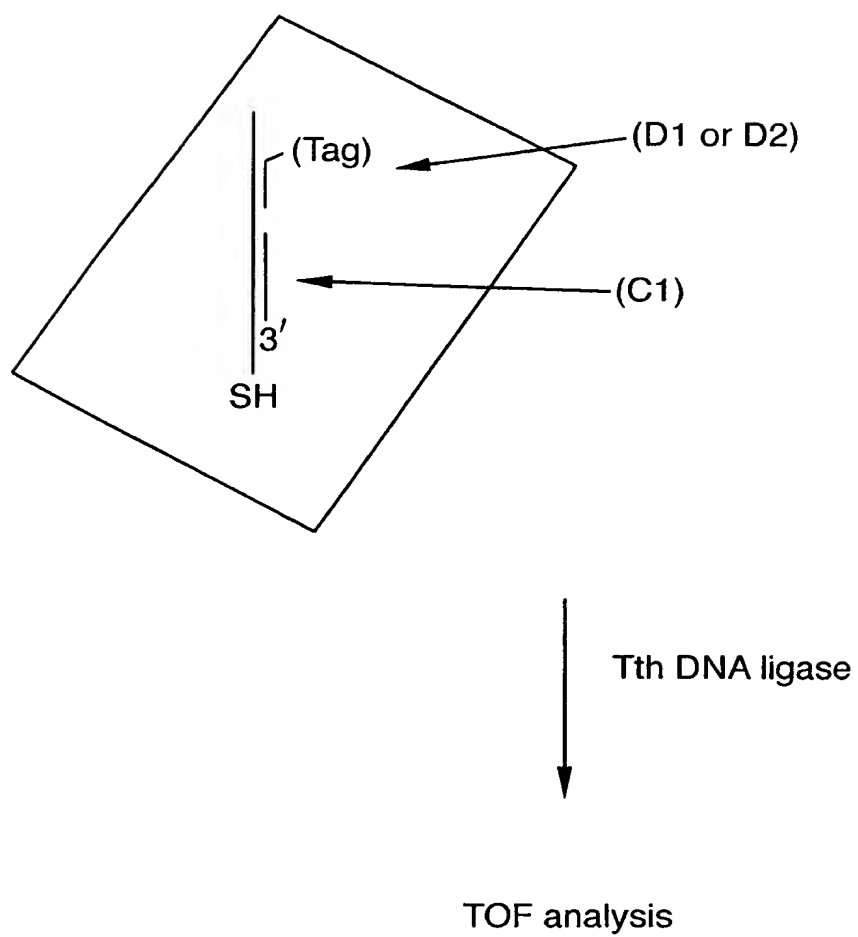
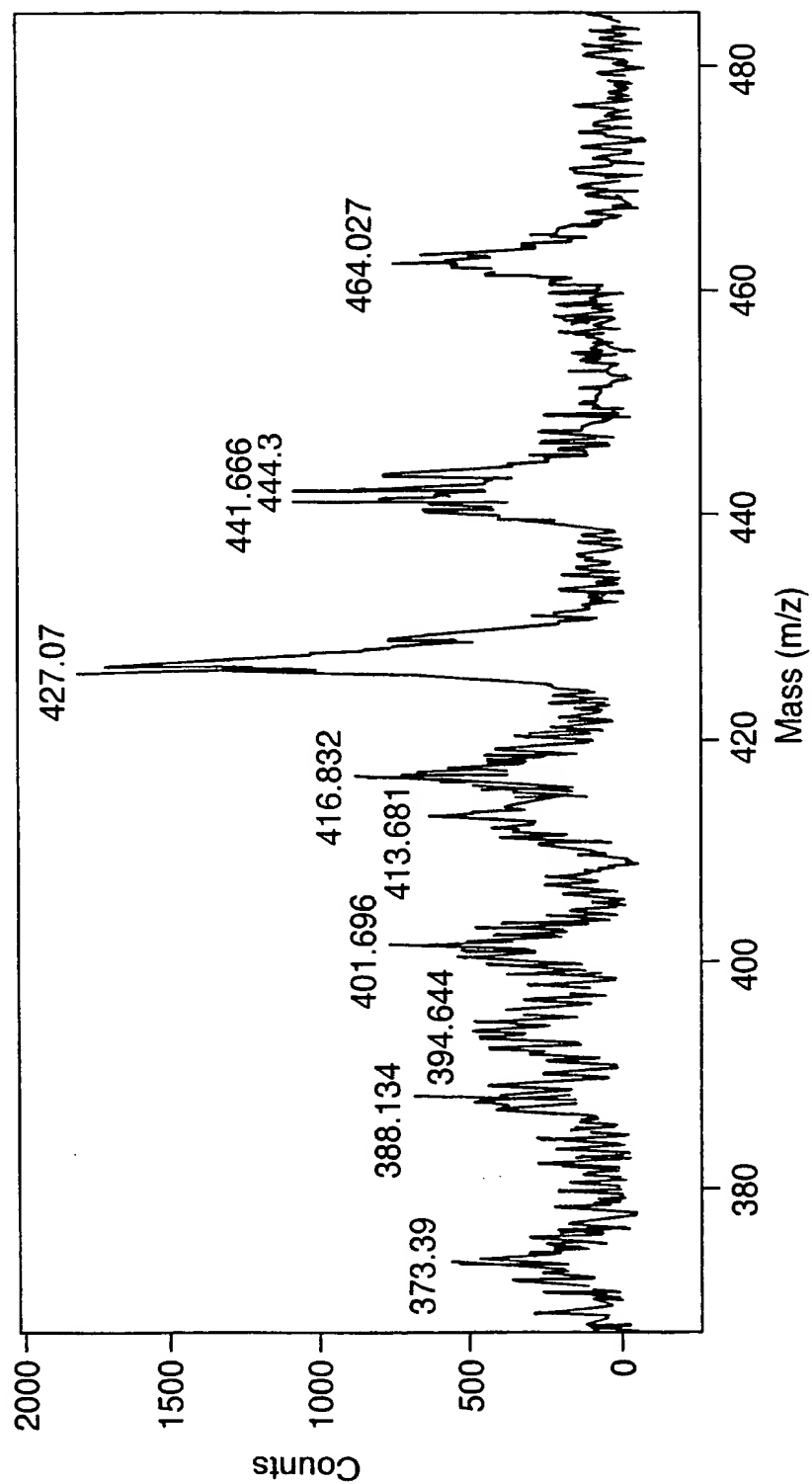


Fig.7.



8/8

Fig.8.

